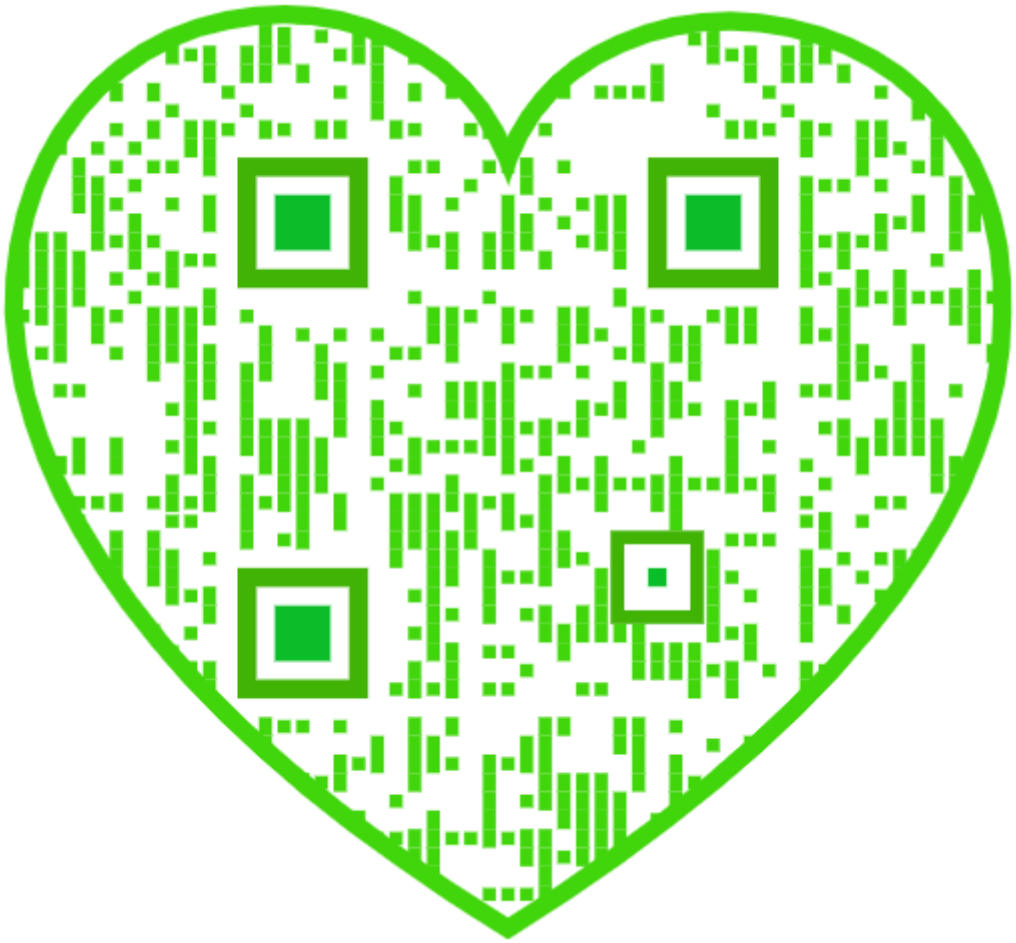

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ANNALS OF BOTANY

EDITED BY

ISAAC BAYLEY BALFOUR, M.A., M.D., F.R.S.

QUEEN'S BOTANIST IN SCOTLAND, PROFESSOR OF BOTANY IN THE UNIVERSITY AND KEEPER OF THE ROYAL BOTANIC GARDEN, EDINBURGH

SYDNEY HOWARD VINES, M.A., D.Sc., F.R.S.

FELLOW OF MAGDALEN COLLEGE, AND SHERARDIAN PROFESSOR OF BOTANY IN THE UNIVERSITY OF OXFORD

D. H. SCOTT, M.A., PH.D., F.R.S.

HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL GARDENS, KEW

AND

WILLIAM GILSON FARLOW, M.D.

PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

ASSISTED BY OTHER BOTANISTS

VOLUME VIII

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ERRATA.

Page 238, last line, *for* 'and formed' *read* 'though they formed'
„ 248, line 12 from bottom, *for* 'tetrach' *read* 'tetrarch'
„ 284, „ 12 „ „ *omit* 'it'
„ 285, „ 14 „ „ *for* 'sexual' *read* 'asexual'
„ 295, „ 21 „ „ *for* 'nuclei' *read* 'chromosomes'
„ 298, „ 5 from top, *for* 'not' *read* 'no'
„ 299, „ 2 „ „ *for* 'with' *read* 'without'
„ 302, „ 13 from bottom, *for* 'alternation' *read* 'alteration'
„ 306, „ 13 „ „ *for* 'small' *read* 'single'
„ 338, „ 17 „ „ *dele* comma after '*margine*'
„ 340, „ 9 from top, for '*Favellae*' read '*Favellis*'
„ „ „ 5 from bottom, for '*frons*' read '*fronde*'
„ „ „ 2 „ „ insert comma after '*minoribus*': *dele* comma after '*tenui*'
„ „ last line, for '*Sphaerosporae*' read '*Sphaerosporis*'
„ 341, line 2 from top, for '*divisae*' and '*immersae*' read '*divisis*' and '*immersis*'
„ „ line 7 from top, for '*vestitis*' read '*vestita*'
„ 342, „ 24 from bottom, *for* 'MYRIOGLOSSA' *read* 'MYRIOPHYLLA'

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Observations on the Development of Marattia Douglasii, Baker.

BY

DOUGLAS HOUGHTON CAMPBELL.

Professor of Botany, Leland Stanford Junior University, California, U.S.A.

With Plates I and II.

WHILE collecting in the Hawaiian Islands during the summer of 1892, I was fortunate enough to find near Hanalei, upon the island of Kauai, a large number of very young plants, of *Marattia Douglasii*, and with them a sufficient number of prothallia with embryos to make it possible to study the principal points in their development. Until Farmer's[1] recent paper on *Angiopteris*, no account of the embryogeny of the Marattiaceae has appeared, except a brief mention made by Luerssen[2] of the arrangement of the primary organs. His material was however too scanty to throw any light upon the early divisions of the embryo. The growth of the prothallium is very slow and the development of the embryo correspondingly late, so that in artificial cultures more than a year must ordinarily elapse before embryos are developed. This probably accounts for the fact

[1] Farmer, On the embryogeny of *Angiopteris evecta*, Annals of Botany, Vol. vi, No. XXIII, Oct. 1892.

[2] Luerssen, Handbuch der Systematischen Botanik, Vol. i, p. 582.

[Annals of Botany, Vol. VIII. No. XXIX. March, 1894.]

that the embryogeny has not been worked out before, as the spores germinate without difficulty.

The Marattiaceae at the present time include four genera, with about 25 species, confined exclusively to tropical regions. In a fossil condition they are much more numerous and diversified than at present, and according to Solms-Laubach [1] comprised the great majority of the Carboniferous and pre-Carboniferous ferns. Their great antiquity, as well as their morphological peculiarities, point to their being primitive forms, and this makes any information concerning their life-history of especial interest. As I have endeavoured to show in several other papers, there is constantly accumulating evidence pointing toward the Eusporangiatae as the forms from which the leptosporangiate ferns have descended, and we shall see that this view is still further strengthened by a study of *Marattia*.

Up to the present time, we have almost no information concerning the embryogeny of the homosporous Eusporangiatae, except Farmer's [1] recent paper on *Angiopteris*, and this being the case, it is hoped that the present paper may have some interest, as adding somewhat to our knowledge of this important group.

Marattia Douglasii is the only representative of the order found in the Hawaiian Islands. It is a large fern with massive leaves, two to three metres in length. It is common in the damp forests at an elevation of 300–400 metres, and occurs upon all the larger islands. The prothallia were found growing thickly upon clay soil near the plants, and were easily distinguished from ordinary fern-prothallia by their fleshy consistence and darker green color. Most of them already had young plants of various sizes attached to them, but a number were found with young embryos. The material was fixed with one per cent. chromic acid, and then gradually transferred to alcohol for study after my return to California.

[1] Solms-Laubach, Fossil Botany, pp. 142-152.

[2] Loc. cit.

Germination of the Spores.

Fresh leaves with ripe sporangia, as well as prothallia and young plants, were collected and were carried back to California, reaching there after more than two weeks confinement in a tin collecting-box, in good condition. Spores were sown and germinated promptly, but the subsequent growth was very slow. No embryos developed from the prothallia, but the latter, as well as the young plants, have grown perfectly well under bell-jars in the laboratory. As the germination of the spores, and the development of the prothallium, has already been exhaustively studied by Jonkman [1], my study of these was mainly confined to the apical growth of the older prothallium, and certain points in regard to the development of the sexual organs. Jonkman showed that the prothallium differs mainly from that of the leptosporangiate ferns by its more massive character. It closely resembles such a liverwort as *Pellia*, for example, having a thick midrib that merges gradually into the thinner wings, which, however, unlike most ferns, are more than one cell thick, except at the very edge. In artificial cultures the growth is very slow, and it may be a year or more before the sexual organs are mature. If fecundation does not occur, the prothallium seems capable of unlimited growth, and may reach a length of two centimetres or more. In the species studied by me, the older prothallia were always decidedly elongated and not orbicular like those of *Angiopteris* described by Farmer [2]. These old prothallia, like those of *Osmunda*, often develop adventitious shoots (Fig. 4 *b*), and in all ways give evidence of almost unlimited capacity for independent growth. In the older prothallia, the midrib is very massive and projects strongly from the lower side where the archegonia are produced in great numbers. The dark green

[1] Jonkman, La génération sexuée des Marattiacées, Arch. Néerlandaises T. XV p. 199.

[2] Loc. cit. p. 265.

colour of the prothallium is much like that of *Anthoceros*, and also recalls strongly, as do the prothallia in other particulars, those of *Osmunda cinnamomea*.

According to Jonkman, the prothallia of the Marattiaceae, like those of the other homosporous ferns that have been investigated, usually at least, grow by a two-sided apical cell which is afterward replaced by a group of marginal initials. In all the specimens at my disposal, the marginal group of cells was already formed and was often wider than is commonly the case in the Polypodiaceae. The form of these initial cells is much the same as in other ferns studied by me. When seen from above (Fig. 1), they are more or less oblong in outline, but in vertical section (Fig. 3), they are nearly hemispherical. As usual, the basal segments are cut off by a wall that extends the whole depth of the prothallium, and the segment is then divided by a horizontal wall into a dorsal and ventral cell of nearly equal size. From the latter, the projecting cushion of tissue on the ventral side is formed. This arises abruptly by the multiplication of cells in the ventral segments some distance behind the apex. The superficial cells of the prothallium, especially upon the upper surface, have a strongly developed cuticle which makes it necessary to use a good deal of care in imbedding the prothallia for sectioning. Numerous root-hairs grow from the lower surface, especially from the midrib, and fasten the prothallium firmly to the ground.

The sexual organs are found in large numbers upon the monoecious prothallia. The antheridia are formed first, and mainly upon the lower surface of the midrib, but also occur upon the upper surface of the prothallium. The archegonia appear later, and so far as my observations go, are confined entirely to the lower surface of the midrib. Upon the old prothallia they may be formed in great numbers, and as they turn dark brown when they fail to be fertilized, are readily visible to the naked eye as dark brown spots thickly studding the broad and thick midrib. Both antheridia and archegonia differ much from those of the leptosporangiate ferns, and the

archegonia, at least, seem to give a possible clue to the origin of the sexual organs of the Pteridophytes.

THE ANTHERIDIUM.

The general structure of the antheridium and its divisions have been so completely described and figured by Jonkman that they will be passed over very briefly here. The antheridium arises from a single superficial cell which first divides into an inner cell, the mother-cell of the sperm-cells, and an outer cover-cell. This latter divides by several curved vertical walls which intersect, and the last wall cuts off a small triangular cell (Fig. 7, *o*), which is thrown off when the antheridium opens and allows the sperm-cells to escape. The inner cell, by repeated divisions, gives rise to a large number of polyhedral cells, the sperm-cells. Before these are completed, however, cells are cut off from the adjacent cells of the prothallium, completely enclosing the mass of sperm-cells (Figs. 7–10). In microtome-sections of material stained with alum-cochineal, the transformation of the nucleus of the sperm-cell into the body of the spermatozoid is easily followed. The nucleus of the sperm-cell, at the time when the division is complete, has the appearance of an ordinary resting nucleus, but no nucleolus is visible. The first sign of the formation of the spermatozoid that could be seen, was an indentation upon one side followed by a rapid flattening and growth of the whole nucleus. The cytoplasmic prominence, which according to Strasburger[1] is the first indication of the formation of the spermatozoid, could not be detected in material stained with alum-cochineal, and although in somewhat later stages (see Fig. 11, *a*) a slight elongation of the forward end of the spermatozoid was sometimes noticed, it was vague and its limits uncertain. The main part of the body stains strongly with alum-cochineal, and for some time shows unmistakably the nuclear structure, and is sharply differentiated against the colourless cytoplasm. In the later

[1] Strasburger, Histologische Beiträge, Heft IV, p. 116.

stages, the body becomes quite homogeneous. In the nearly full-grown spermatozoid, the body is much narrower and tapers to a fine point at the forward end (Fig. 12). From a careful study of all stages, it seems certain that only a very small part of the forward end is of cytoplasmic origin. Unfortunately, I was unable to get any free spermatozoids, so that it was impossible to test them with Strasburger's [1] and Belajeff's [2] methods, but in the full-grown spermatozoid within the antheridium the nuclear origin of all but the extreme forward end was unmistakable. Perhaps, as Strasburger claims, the cilia-bearing part is cytoplasmic and the cilia are direct outgrowths of this part, but this point was not satisfactorily proven. The origin of the antheridium, as well as its development and its complete immersion in the prothallium, have their nearest approach in *Equisetum*. The species of *Lycopodium* investigated by Treub [3] also show a good deal of resemblance. Among the ferns, from what is known, the Ophioglosseae show a close resemblance, but the development of the antheridium is too imperfectly known to allow of a satisfactory comparison. The antheridium of *Osmunda* is in some degree intermediate between that of Marattiaceae and the more specialized Leptosporangiatae.

The Archegonium.

The archegonia seem to be confined to the lower side of the midrib, and are formed some distance behind the growing-point of the prothallium. So far as could be determined, any superficial cell of the apical meristem can develop into an archegonium. The succession of divisions seem to correspond exactly with those of the other ferns. The mother-cell divides usually into three superimposed cells (Fig. 13), of which the lowest, *b*, usually divides several times by vertical walls and forms the base of the archegonium. From the central one, by transverse divisions are formed the canal-cells and egg,

[1] Loc. cit. p. 106.

[2] Belajeff, Ber. der Deutschen Bot. Gesellschaft, Dec. 1889.

[3] Annals of the Botanical Garden, Buitenzorg, Vols. iv, v, vii.

and from the uppermost the neck. Compared with the homosporous leptosporangiate ferns, the most striking differences are the short neck and the broad canal-cells. The neck projects but little and contains only three or four cells in each row. The egg-cell is small and the ventral canal-cell extraordinarily large. Jonkman[1] has shown that the neck canal-cell is frequently divided, and Farmer[2] has confirmed this as a common but not invariable occurrence in *Angiopteris*. In *Marattia Douglasii*, it does not ordinarily occur, although the division of the nucleus seems always to take place. While a trace of a wall was not infrequent, in no case was a firm wall, such as Jonkman showed, to be seen. As the number of perfect sections of mature archegonia was small, however, it is not at all unlikely that in this species, too, a complete division of the neck-cell may often occur. Of the Filicineae, *Isoëtes* comes nearest to *Marattia*, from which it differs mainly in the larger size of the egg and the absence of the basal cell of the archegonium.

The study of the archegonium of *Marattia* has suggested a possible explanation of the origin of the archegonium of the Pteridophytes which is so constant in its structure, and seems to differ so radically from the bryophytic type. All of the eusporangiate Pteridophytes are characterized by the short neck of the archegonium, while the leptosporangiate ferns have as a rule a relatively long neck; but in all, the base of the archegonium is never free. Among the Bryophytes, with the single exception of the Anthoceroteae, the young archegonium is entirely free and the first divisions are always the same. In the Anthoceroteae, the archegonium is even more completely sunk in the thallus than in any Pteridophyte. It was shown by Janczewski[3] and Leitgeb[4], however, that the archegonium of *Anthoceros* does not differ so much from that of the other Liverworts as has been supposed, and that

[1] Loc. cit. p. 216.
[2] Loc. cit. p. 266.
[3] Janczewski, Bot. Zeitung, 1872.
[4] Leitgeb, Untersuchungen über die Lebermoose, Heft V, pp. 3, 4.

the axial row of cells, which Hofmeister[1] regarded as the whole archegonium, really represents only the central row of the typical bryophytic archegonium, as this axial row of cells is cut out by walls which correspond to those in the archegonium-mother-cell of other Bryophytes. It seems to me from a careful study of *Marattia*, as well as other ferns, that this is the case in the Pteridophytes, and that what is ordinarily called the mother-cell of the archegonium, is homologous, not with the mother-cell of the archegonium of a Liverwort or Moss, but with the central row of cells only. The cells which are later cut off from the adjacent prothallium-cells (Fig. 16–17, *m*) probably represent the peripheral cells of the archegonium. The neck of the archegonium, too, here seemed to be of a totally different type. In all Pteridophytes it is composed of four rows of cells, derived from the cross-divisions of the outermost of the three original cells. Examining the Bryophytes, we find two distinct types of archegonium, that of the Liverworts and that of the Mosses: while in both the neck is composed of six, occasionally five, outer rows of cells, in the Liverworts at a very early stage the upper cell of the axial series divides by cross-walls into four and forms the upper cells of the neck: in the Mosses, on the contrary, the upper cell functions for a long time as an apical cell before its final cross-division, and adds new cells both to the outer cells and to the canal-cells. Now the question is, how was the 4-rowed neck of the pteridophytic archegonium derived from the 6-rowed neck of that of the Bryophytes? The answer probably is, that it represents a development of the four terminal cells only. These in Liverworts usually undergo a single division, or occasionally more than one. If we accept this view, it will bring still closer together the eusporangiate ferns and the Anthoceroteae, and the latter will become of still more importance in the study of the phylogeny of the ferns. We shall then assume that the Eusporangiatae are derived from Liverworts in which, as in *Anthoceros*, the archegonium was completely sunk in the thallus, and that from the four

[1] Hofmeister, Higher Cryptogamia, p. 9.

primary cells of the cover-cell of the neck, by further divisions, have arisen the four series of cells that form the neck in these forms. It is probable that this increase in length is in some way connected with the fertilization, as is the backward curvature of the neck in the more specialized leptosporangiate forms.

Fertilization.

The entrance of the spermatozoids into the archegonium was not seen, but in a number of cases the material used had been killed immediately after, as several times the spermatozoid was found within the egg. The mature egg was in most cases somewhat contracted during the process of imbedding, but in a few cases (see Fig. 18) it retained its normal form. It was then seen to be slightly elliptical, the upper third being nearly homogeneous and quite colorless, forming the receptive spot. The nucleus is of moderate size, and not rich in chromatin; a small but distinct nucleolus is present. In two cases the spermatozoid, quite unchanged, was seen within the recently fertilized egg. In both cases the spermatozoid was strongly stained and was extremely conspicuous in the protoplasm of the egg, which was only slightly granular and entirely colourless. In both of these, the spermatozoid had penetrated the egg completely, and its pointed end was in direct contact with the membrane of the egg-nucleus. In what seemed a later stage (Fig. 20), two nuclei in close contact were seen, but the female nucleus seemed much contracted: whether this was the normal condition previous to the fusion of the nuclei I cannot say, as no others were found in this condition. In a still older one (Fig. 21), where the membrane of the fertilized egg was very evident, and several spermatozoids were noticed about it, the nucleus showed two nucleoli, and a quite distinct division line, showing that the identity of the two conjugating nuclei had not been lost.

The material at my command was not sufficiently abundant to warrant any attempt to follow out completely the process of fertilization, but from the few preparations where the

conjugating nuclei were seen, the clearness with which the nuclei were differentiated from the colourless cytoplasm indicates that this would prove a very satisfactory plant for studying fertilization.

The Embryo.

I was not fortunate enough to get satisfactory preparations of the earliest stages of the embryo. A number of single-celled embryos were seen, but the following stages were not found. This much can be said, however:—after fertilization, the egg enlarges to several times its original size before dividing, and the first wall is parallel to the surface of the prothallium, as in *Angiopteris*, and as is also the case in *Isoëtes* and *Equisetum*. This is followed by the median and octant walls, but in what order it is impossible to say.

The youngest embryo found is shown in Figs. 22 and 23. In this the basal and median walls were very plain, and several divisions had appeared in the octants. Compared with other ferns there is less regularity in the arrangement of the early division-walls in the octants, and this is undoubtedly associated with the much less definite growth in the different members of the young plant. Still, the regular formation of octants, and the lateral segments cut off from these before any periclinal walls are formed, probably indicates that at first, as in the mature organs of most ferns, the growth is from a single tetrahedral cell. In the next older embryos found (Figs. 24 and 25) the primary divisions had already ceased to be certainly distinguishable, and the limits of the octants could no longer be positively determined. As in other ferns, the first or basal wall determines the position of the primary organs, the cotyledon and stem-apex arising from the epibasal cell, the root and foot from the hypobasal. The Marattiaceae differ, however, from all the known forms in the position of these organs with reference to the archegonium. Whereas in all other forms yet investigated the cotyledon is derived from one of the quadrants adjacent to the neck of the archegonium, in *Marattia* (and Farmer has shown this to be

true of *Angiopteris*, as well) the cotyledon originates from one of the inner quadrants, and as we shall see, grows up through the prothallium instead of from the under side, as in other ferns. The young embryo retains a nearly oval outline even after it has reached a considerable size, and the external differentiation of the members arises much later than is usually the case. This lateness in the development of the organs is correlated with a correspondingly late differentiation of the primary tissue-systems.

As might be expected, *Marattia* agrees in the early embryonic stages most nearly with the closely related *Angiopteris*. *Osmunda* comes nearest it among the other ferns in the late establishment of the primary organs and tissues, and in both these respects, seems to stand between the Marattiaceae and the true Leptosporangiatae. *Isoëtes* resembles *Marattia* quite closely in the early divisions of the embryo and the absence of a definite apical cell in the cotyledon; but the position and origin of the stem-apex of *Isoëtes* differ from all other Pteridophytes, and have their nearest approach among the Monocotyledons.

The Cotyledon.

The cotyledon arises from the anterior pair of epibasal octants, and in the earlier stages seen showed no definite apical cell. The growth is at first nearly vertical, but very soon growth on the upper side is much stronger and the leaf becomes strongly bent over. Vertical sections of an embryo at this stage show that the tissues are already beginning to be distinguishable (Fig. 29). A little later the conical cotyledon becomes flattened towards the end so that the lamina becomes differentiated, and this is accompanied by a dichotomy of the lamina, which is repeated (Fig. 34), so that at the time the young plant breaks through the prothallium the cotyledon is more or less strongly bilobed and the divisions are also lobed. This is accompanied by a forking of the veins, so that in the primary leaf, as well as the later ones for some time, the branching of the lamina is

strictly dichotomous, and it is only gradually that the monopodial branching is established. Farmer[1] shows in his figures of *Angiopteris* the cotyledon of a more spatulate form with a distinct midrib and lateral veins, and in this respect *Angiopteris* would appear to differ more from *Marattia* than the latter does from most of the Leptosporangiatae where the dichotomous branching of the lamina is nearly constant. The nearly cylindrical petiole is deeply channelled upon the inner side and the single central vascular bundle is almost circular in section. While the crescent-shaped mass of tracheary tissue is completely enclosed by the phloëm (Figs. 38–39), the development of the phloëm is much less upon the inner side, and the bundle approaches closely the collateral type.

The tannin-cells, which according to Farmer's account are so conspicuous in *Angiopteris*, are also found here, but are not so marked. These are the shaded cells in Fig. 39. If these belong to the cortex, as Farmer asserts, this would bring the xylem of the bundle into direct contact with the ground-tissue, and consequently the bundle would be truly collateral. This point was not however investigated by me.

The lamina of the cotyledon is similar in structure to that of the later leaves, differing mainly in the much smaller development of the mesophyll. The smaller veins have the xylem reduced to a few (1–3) small tracheids which are situated upon the upper side of the bundle. Stomata of the ordinary form (Figs. 35–36) occur upon the lower surface of the leaf.

The Stem.

In the older embryos, the apex of the stem (Figs. 30–33) is occupied by a group of relatively large cells, which at first seem to be pretty nearly alike; but a careful examination of these led me to think that only one or two of these are to be regarded as properly initial cells. In the cross-section shown in Fig. 33, for instance, the arrangement of cells is such as to

[1] Loc. cit. Figs. 19–21.

indicate that from the cell x, segments were cut off in regular succession. In other cases, however (see Fig. 31), such regularity is not apparent, and two initials (x – x́) can be seen. Unfortunately so few of the very young embryos were found that it cannot be stated now what is the condition in the earlier stages, but it is not impossible that sometimes, at least, one of the primary octants persists as the apical cell of the stem of the young plant. In *Angiopteris*, Farmer could not detect a single initial for the stem in the young plant, and Bower [1] describes a group of initials in the stem-apex of the older plant in his later publication, although attributing but a single one in his first investigation [2] of the plant.

The Root.

In the root there seems to be much the same variation that exists in the stem. Here again my observations were of necessity confined mainly to a study of the older embryos. Here there is, usually at least, but one initial cell, as in other ferns, but it is very variable in form. There seems no reason to doubt that, as in these, it can be traced back to one of the primary hypobasal octants. In *Angiopteris*, Farmer found in the very young embryos a regular triangular apical cell, and very likely such a one exists in *Marattia* also, but none of my preparations of the younger embryos were cut so as to show this. An approach to this was seen in transverse sections (Fig. 41) where three sets of lateral segments could be made out; but in longitudinal sections, the initial cell appeared usually more or less regularly four-sided, and in the case shown in Figs. 42–44, although the cross-section of the apical cell appears nearly triangular, a section further down shows unmistakably that there are four sets of lateral segments. The segments are larger and the divisions show much less regularity than is found in the leptosporangiate

[1] Bower, Comparative examination of the meristems of ferns, Ann. Bot. III, pp. 324. 325.

[2] Bower, Comparative morphology of the leaf, &c., p. 579: Phil. Trans., Royal Society, 1884.

type. When the base of the apical cell is triangular (Fig. 40), the segments are cut off from the base, and these take part in the formation of the plerome-cylinder. The root-cap arises in part from the outer segments of the apical cell, but is formed also in part from the outer cells of the newly formed segments. In later roots a single apical cell is not always certainly to be seen, and it is probable that as the roots increase in size one or more of the segments of the apical cell assume the function of initials and thus a group of initial cells is formed that replaces the original single apical cell.

The vascular cylinder of the root is usually tetrarch. At four points in the periphery small spiral or annular tracheids arise, and from them the formation proceeds as usual toward the center of the bundle. The phloëm is made up of nearly uniform cells with moderately thick colourless walls. The bundle-sheath is much less definite than is usual in ferns.

The foot is much less prominent than is usual, and its limits are not at all clearly defined. In fact all of the superficial cells of the central region of the embryo become enlarged and appear to act as absorbent cells.

As the embryo grows, the surrounding prothallial cells divide rapidly, so that the young plant is completely enclosed within a sort of calyptra for an unusually long time. Owing to the position of the cotyledon and stem, which are turned toward the upper surface of the prothallium and grow vertically, a very conspicuous elevation is formed upon its upper side, and finally the cotyledon bursts through this and appears upon the upper surface of the prothallium. The root grows downward, its axis almost coinciding with that of the cotyledon; but it does not break through the prothallium until the cotyledon has nearly reached its full development. This fact is especially significant, as, in the leptosporangiate ferns, the root is usually the first organ of the young plant to break through, and is fastened in the ground before the cotyledon is visible from the outside. Of the other ferns,

Osmunda again shows in the relative time of emergence of cotyledon and root, a condition intermediate between the Marattiaceae and the higher leptosporangiate forms. The peculiar method of emergence of the embryo of the Marattiaceae was first described by Luerssen[1]. If we admit the primitive nature of the Marattiaceae, this peculiarity may probably be regarded as an indication that the ancestral forms had the archegonium upon the upper side of the prothallium, as in *Anthoceros*, but that with the shifting of the archegonium to the lower side, probably to insure fertilization, the embryo has retained its original position with respect to the prothallium, while in the leptosporangiates this has been changed.

There is nothing peculiar in the development of the vascular bundles. As usual the first tracheary tissue arises at the junction of the bundles of the cotyledon, stem and root, and proceeds toward the apices of these organs. Short hairs, with the cells rich in tannin, which stain very deeply with Bismarck-brown, occur sparingly upon the leaves and stem of the young plant.

One of the most interesting points brought out during this investigation was the extraordinary persistence of the prothallium. Not only does this grow indefinitely when it remains unfecundated, but even after the young plant has broken through, the prothallium remains fresh and green for a long time. The plant shown in Fig. 46 must have been nearly a year old, as it was collected in August, 1892, and the drawing was made in May 1893, and not more than two new leaves had formed in the meantime: yet the prothallium, although torn apart by the growth of the young plant, was still green and fresh[2].

[1] Loc. cit. p. 582.

[2] Since the above was written the writer was fortunate enough to find a number of young plants of *Botrychium virginianum* with the prothallium still attached. These were subterranean, but larger than those of *B. Lunaria*, to judge from Hofmeister's account. Although the sporophyte was in some cases as much as ten centimetres high (including the underground portion), the prothallia were still fresh, and in some cases, at least, bore fresh antheridia.

The first leaf is destitute of the stipules characteristic of the leaves of the older plants, but the third leaf has them well developed. The second root arises close to the base of the second leaf and at first there seems to be a root formed at the base of each of the young leaves. In the older sporophyte the roots are more numerous.

It has been shown that in the earliest stages the divisions of the embryo of *Marattia* correspond with those of the other Pteridophytes and that the position of the primary organs is the same with reference to the basal wall, but that their position with reference to the archegonium is different. In all other forms the cotyledon arises from one of the quadrants next to the neck of the archegonium, while the reverse obtains here. Probably the transverse basal wall is the primitive condition, as it is found in all Eusporangiatae examined and is also found in nearly all Bryophytes. The different arrangement in most Leptosporangiatae is probably due largely to the shifting of the position of the archegonium. Of other ferns we have seen that, next to *Angiopteris* with which *Marattia* closely agrees, *Osmunda* most nearly resembles it. *Isoëtes*, while showing a resemblance in the early divisions of the embryo, differs very much, not only from the Marattiaceae, but all other Pteridophytes, in the origin of the stem-apex, which resembles closely that of some Monocotyledons. With the higher Leptosporangiatae the embryo of *Marattia* has little in common beyond the first divisions.

CONCLUSIONS.

From a careful study of the facts presented here, as well as the other recent contributions to our knowledge of the Marattiaceae, there seems to be no reason to modify the opinion already several times expressed, of the primitive nature of the eusporangiate Pteridophytes. These, however, as well as a careful study of several species of *Anthoceros*, will enable us to understand somewhat more closely the position of the Marattiaceae in the system, and the origin of the Eusporangiatae from the Bryophytes.

The very massive, long-lived prothallium of *Marattia*, and especially the fact that the prothallium remains active long after the sporophyte becomes independent, seem conclusive evidence of the primitive nature of the former. The long dependence of the embryo also, and the late development of the root, point to the same thing. In both of these particulars *Marattia* surpasses all other Pteridophytes known. It is quite possible that the Ophioglosseae may show similar peculiarities, but they are too imperfectly known to determine this. The peculiar position of the embryo, also, points to the not very remote derivation of these plants from those in which the archegonium was upon the upper surface of the prothallium, as, in all other forms where the archegonium is upon the lower side, the basal wall has shifted in such a way as to bring the cotyledon next to the neck of the archegonium and it emerges below. In all of these particulars, as well as in the structure of the archegonium, *Marattia* comes nearest to the Liverworts of any Pteridophytes yet examined, and of the Liverworts, the Anthoceroteae are undoubtedly the ones to which the resemblance is most marked. This peculiar group has been the subject of repeated exhaustive researches, and yet some of the most striking points of resemblance between them and the Pteridophytes seem to have been overlooked. The remarkable correspondence between the archegonium of *Anthoceros* and that of *Marattia* has already been referred to, and gives a possible explanation of the origin of the pteridophytic archegonium from that of the Bryophytes. The antheridium, however, is not so easily explained. All of the eusporangiate forms have the antheridium also sunk in the prothallium, and its origin in *Anthoceros* is still an open question[1]: but in the latter, whether endogenous in origin or not, the mature antheridium is closely like that of the other Liverworts. There is, however, a close resemblance between the antheridium of *Marattia* and that of the other

[1] Since writing the above my attention has been called to a paper by M. Waldner (Die Entwickelung des Antheridiums von Anthoceros, Sitzungsb. der Kais. Akad. der Wiss. LXXXV, Wien, 1887), but unfortunately the paper was not accessible.

eusporangiate Pteridophytes. In all of these it is usually completely sunk in the tissue of the prothallium and its dehiscence is much the same. The archegonium also in these other forms has the short neck of Marattiaceae. From a study of these facts the inference may be drawn, that the ancestral forms had a relatively massive prothallium with the sexual organs upon the upper side, and that they were completely sunk below the surface as in *Anthoceros* and *Marattia*; further, that the projecting neck of the archegonium and the peculiar antheridium of the Leptosporangiatae are secondary characters correlated with the pecularities in the prothallium of these forms. This, of course, implies that the filamentous prothallium characteristic of certain species of *Trichomanes*, and found occasionally in other ferns, is a secondary and not a primary character.

In regard to the sporophyte, it will not be necessary to repeat what has already been said in a former paper as to a comparison of the sporophyte of *Anthoceros* with that of *Ophioglossum*. One additional point, however, may be brought out. At an early stage in the development of the embryo of *Anthoceros* the capsule-wall is separated from the central tissue, and by a subsequent division of this outer layer of cells, the sporogenous tissue is cut off. That is, the archesporium is derived from hypodermal cells exactly as is the case in the eusporangiate Pteridophytes, but this does not occur elsewhere among the Bryophytes.

Like the Osmundaceae, the Marattiaceae seem to stand near the junction of several divergent lines of development. Related on the one hand to the Ophioglosseae, they show unmistakable resemblance to the Osmundaceae, and possibly to *Equisetum*. It has already been suggested that *Isoëtes* also belongs to the same stock, and that through forms like it the Angiosperms may have arisen. If this hypothesis should prove to be correct, *Marattia* must be regarded as one of the earlier forms in which the single initial cell in the different members is being replaced by the group of initial cells found in most Angiosperms.

Owing to the very small number of the eusporangiate Filicineae now existing, we can never hope to trace out an unbroken line of descent, but when the numerous fossil remains have been as carefully studied as the living ones, much additional information bearing on the subject may be reasonably expected.

EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Professor Campbell's paper on *Marattia*.

PLATE I.

Fig. 1. Horizontal section of the apex of a small prothallium with two initials, x, x'. × 300.

Fig. 2. Vertical section of the apical region of a prothallium; *ar*, archegonium; x, one of the apical cells. × 50.

Fig. 3. Apical region of a similar section. × 300. x, apical cell; *d*, dorsal segment; *v*, ventral segment.

Fig. 4. An old prothallium with adventitious buds *b*. × 2.

Figs. 5–7. Surface views of antheridia in different stages. In Fig. 7 the dotted lines indicate the lateral cells; *o*, opercular cell. × 300.

Figs. 8–10. Vertical sections of young antheridia. × 300.

Figs. 11, 12. Young spermatozoids. × 1200.

Figs. 13–16. Development of the archegonium. 13, 14 × 600: 15, 16 × 300, *b*, basal cell; *o*, egg; *c*, neck-canal-cell; *v*, ventral canal-cell; *n*, neck; *m*, peripheral cells.

Fig. 17. Cross-section of the base of the archegonium. × 300.

Fig. 18. Lower part of archegonium with ripe egg. × 650.

Fig. 19. Recently fertilized egg showing the spermatozoid in contact with the egg-nucleus. × 650.

Fig. 20. A later stage. × 650.

Fig. 21. Fertilized egg showing almost completed fusion of the sexual nuclei. × 600.

Fig. 22. Vertical section of young embryo. × 300. *b b*, basal wall.

Fig. 23. Diagram of the same embryo, *b b*, basal wall; II II, transverse wall.

Fig. 24. Vertical section of an older embryo. × 300. The arrow indicates the position of the archegonium.

Fig. 25. Nearly median transverse section of an older embryo. × 300.

Fig. 26. A still older embryo in nearly median vertical section. × 100. *l*, cotyledon; *st*, stem; *r*, root; *ar*, archegonium.

Figs. 27, 28. Prothallia with young plants. × 2.

PLATE II.

Fig. 29. Median section of the cotyledon of an embryo of the same age as the one shown in Fig. 26. × 300.

Fig. 30. Vertical section through the stem-apex of a young plant in which the second root r^2 was already formed; *x*, initial of stem; *tr*, first tracheids. × 250.

Fig. 31. Transverse section of stem-apex with two initials, *x x'*. × 600.

Fig. 32. Transverse section through the base of the cotyledon and stem-apex. × 100.

Fig. 33. A stem-apex of the same embryo. × 300. *x*, the single initial cell.

Fig. 34. Horizontal section of the lamina of the cotyledon. × 300.

Figs. 35, 36. Stomata. Fig. 35 surface view. × 300. Fig. 36, vertical section. × 600.

Fig. 37. Cross-section of the lamina of a nearly full-grown cotyledon, passing through one of the smaller veins. × 300.

Fig. 38. Cross-section of the petiole of the cotyledon. × 50.

Fig. 39. The vascular bundle. × 300.

Fig. 40. Longitudinal section of root-apex. *x*, the initial cell. × 300.

Fig. 41. Transverse section of the apical meristem of the root. × 300.

Figs. 42–44. Three cross-sections of a root from a young plant. × 250. Fig. 42, the apical meristem; Figs. 43, 44, sections taken lower down.

Fig. 45. Cross-section of fully developed vascular bundle of the root. × 300.

Fig. 46. Young plant with persistent prothallium *pr*; natural size.

Fertilization of Pinus silvestris [1].

BY

HENRY H. DIXON, B.A.

Assistant to the Professor of Botany in Trinity College, Dublin.

With Plates III, IV, and V.

PREVIOUSLY to the appearance of Belajeff's paper in 1891 on the behaviour of the nuclei of the pollen-grains of *Taxus baccata* [2], it was believed that the nucleus of the pollen-tube of the Gymnosperms was the male sexual nucleus, and that the cell-group formed in the pollen-grain opposite the point of exit of the pollen-tube was composed of two or more asexual cells corresponding to the sterile cell cut off in the microspore of the Selaginelleae. Prof. Strasburger had already shown [3] that the nucleus of the pollen-tube of Angiosperms was asexual, and that although it passed into the tube it eventually disintegrated and took no part in fertilization; while the nucleus of one of the cells originally behind the nucleus of the pollen-tube performed the function of fertilization. Belajeff, in the paper already referred to, showed that the nuclei of the pollen-grains of *Taxus* behaved in a manner resembling in its most important points that of the Angiosperms: i. e. that the nucleus of the pollen-tube is asexual and that fertilization takes place by the union of one of the nuclei of the two cells resulting from the division of one

[1] This research was carried out in the Botanisches Institut of the University of Bonn, at the suggestion and under the supervision of Professor Strasburger, who not only supplied me the necessary material, but also was good enough to give me the benefit of his great experience.

[2] Berichte der Deutschen botanischen Gesellschaft, Bd. IX, p. 280.

[3] Befruchtungsvorgang bei den Phanerogamen. Jena, 1884.

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of the cells of the cell-group formed within the pollen-grain. This fact, which was established by Belajeff for *Taxus baccata*, was shown to be generally true for the Gymnosperms by Prof. Strasburger [1].

The misunderstanding with regard to the nature of the cell-group of the pollen arose from the fact that in all artificial cultures the cells composing it remain in the position where they are formed, while the nucleus of the pollen-tube approaches the end of the tube. Accordingly it was naturally supposed that the cell-group could not contain sexual nuclei as, in the cultures, its nuclei never left the pollen-grain and consequently could not reach the oosphere; whereas the nucleus of the pollen-tube, by wandering into the tube, would naturally be in a position to unite with the female nucleus. And hence it was regarded as the sexual nucleus. Belajeff found however, that, when on the nucellus, the cells of the included cell-group of the pollen of Gymnosperms behave quite differently.

Prof. Strasburger found that in some cases (e. g. *Larix europaea*) several divisions had already taken place in the pollen-grain before it had reached the nucellus. In this instance the undivided pollen-grain divides while still in the anther into a large and a small cell [2]. The latter is lens-shaped and applies itself closely to the wall of the pollen-grain. Its contents soon become highly refractive and it flattens itself against the wall of the pollen-grain. About this time another cell is cut off from the large cell and it applies itself against the first cell, which is already flattened against the wall. This second cell suffers the same fate as the first, and then a third much more strongly arched cell is cut off from the large cell. This third cell is so placed that it covers over the first two cells, which by this time are completely flattened out and appear as mere cracks in the wall of the pollen-grain. Unlike the two first-formed cells, this cell does not flatten out, but divides into two, a small stalk-cell

[1] Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. Jena, 1892. Belajeff also records further observations of his own in Ber. der Deut. Bot. Ges., April 26, 1893.

[2] Befruchtungsvorgang bei den Phanerogamen. 1884, p. 2.

(Stielzelle) next the wall of the pollen-grain and a larger body-cell. Where the cell-group is, in this way, composed of more than two cells, Prof. Strasburger found that it was already formed in the anther; whereas in others (e. g. *Taxus*, *Cupressus*, &c.) the pollen-grain reached the nucellus before any cell-division took place within it.

As the result of these divisions, which have taken place in the pollen-grain either while it is still in the anther, or after it has been transported to the nucellus, there are in it three cells; the large cell which grows out to form the pollen-tube, the small stalk-cell, and the body-cell, which by a subsequent division gives rise to two cells, which contain the male sexual nuclei. Besides these cells, there are in *Larix* and most of the Coniferae, in which several prothallium-cells are formed, the remains of these prothallium-cells persisting as scarcely perceptible clefts in the wall of the pollen-grain at the base of the stalk-cell.

It was by tracing the subsequent behaviour of this cell-complex formed within the pollen of *Taxus*, that Belajeff showed that the nucleus of the pollen-tube was not a sexual nucleus, but that fertilization was effected by the nucleus of one of the cells arising by the division of the body-cell. According to the further researches of Belajeff and Prof. Strasburger, after the pollen-tube is formed and its nucleus has moved into it, the body-cell breaks free in the pollen-grain and wanders into the tube following the nucleus of the tube. This breaking free of the body-cell appears to be facilitated by the fact that the stalk-cell relinquishes its independence and through the rupture in its wall, left by the breaking away of the body-cell, its nucleus escapes and follows the body-cell into the pollen-tube. The nucleus of the stalk-cell soon overtakes the body-cell and, passing it by, comes into a position close to the nucleus of the pollen-tube. In all cases so far examined, the body-cell divides into two daughter-cells either while yet in the pollen-grain or after it has passed into the pollen-tube. An example of the first case is *Larix europaea*, and of the second are *Taxus baccata* and *Juniperus virginiana*.

In most cases the sister-cells arising from this division are almost of the same size as in *Biota*, *Juniperus*, *Ginkgo*, and the Abietineae; whereas in *Taxus baccata* they are very unequal in size. It is usual in *Biota* and *Juniperus* for these two cells to lie side by side in the end of the pollen-tube, but in *Taxus* the larger one is usually below the smaller and closer to the end of the tube. When the nucleus of the stalk-cell has passed by the body-cell or its two daughter-cells and taken up its position near the nucleus of the pollen-tube, it becomes extremely similar to this nucleus. This similarity is probably due to the fact that the two nuclei are nourished by the same cytoplasm.

The history of the nuclei of the pollen-grains of the Abietineae had not been yet so exactly traced as in other groups, and accordingly it was hoped that some points still unobserved, and with regard to which surmises only had been formed, might be made out by investigation in this group. For the investigation Prof. Strasburger was kind enough to supply me with pollinated cones of *Pinus silvestris* taken at intervals of a week, dating from April 24 to June 6, 1893. The cones, of course, were pollinated in 1892, as the pollen-grain of *Pinus silvestris* reaches the nucellus about thirteen months before fertilization takes place.

Prof. Strasburger had already determined that, in the ripe pollen-grain of *Pinus silvestris*, there are a small prothallium-cell, the last of three formed, and a large nucleus. This latter is the nucleus of the pollen-tube and passes into the tube which is formed immediately after pollination. The prothallium-cell remains attached to the wall of the grain, opposite the place of exit of the pollen-tube. In this condition the pollen remains during the winter.

The following research is concerned with the subsequent history of the pollen.

The next spring the pollen-tube becomes filled with starch, and, towards the end of April, the prothallium-cell divides to form a small stalk-cell and a larger body-cell. Pl. III, Fig. 1 shows this division just completed. Very shortly after this

it is found that the body-cell has broken free from the stalk-cell and has divided into two cells, which are almost equal in size (Figs. 2, 3). These cells are the male sexual cells. During this process the wall of the stalk-cell is ruptured and its nucleus follows the two cells resulting from the division of the body-cell which move into the pollen-tube (Fig. 4). The wall of the stalk-cell is ruptured in such a manner that there remains a ring-shaped portion of it standing up from the inner wall of the pollen-grain. This persists for a long time and may still be seen in much later stages, as in Fig. 10.

The two male cells move slowly down into the pollen-tube and are closely followed by the nucleus of the stalk-cell (Fig. 5), which ultimately overtakes them. Fig. 6 shows the nucleus of the stalk-cell just passing the two sexual cells. In the section from which Fig. 7 is drawn, the nucleus of the stalk-cell is well past the sexual cells and all three are wandering down the pollen-tube. The same relative position is shown in Fig 8. As these three, the two sexual cells and the naked nucleus of the stalk-cell, pass down the pollen-tube and finally approach its lower extremity, they naturally come into a position close to the nucleus of the pollen-tube. This latter nucleus at this stage is usually very different in appearance from the nucleus of the stalk-cell; in fact, it resembles the nuclei of the sexual cells, its nucleolus appearing like a very refractive ring, while the nucleus of the stalk-cell is coarsely granular (Fig. 9). I did not find the sexual nuclei so close to the lower end of the pollen-tube as is shown in Fig. 9 till May 12. As soon as the nucleus of the stalk-cell leaves the pollen-grain and passes into the pollen-tube, the former is usually more or less completely emptied of its contents. In this condition it is often very easy to see the ring-shaped remnant of the wall of the stalk-cell, Fig. 10. Fig. 11 shows the remains of two prothallium-cells as well as the ring-like wall of the stalk-cell.

During the period between April 26 to May 12, the growth of the pollen-tubes was found to be extremely slow, their passage being made through the hard tissue at the top of the nucellus,

the cells of which are provided with brown cell-walls. These cells do not contain nearly so much starch as the cells beneath, which have not brown cell-walls and which are much softer to cut; but even while penetrating this upper part the pollen-tube is often so completely filled with starch that it is very difficult to see the nuclei within it. The pollen-tube penetrates the nucellus, exercising a destructive influence on the cells in its immediate neighbourhood. These cells lose their nuclei and become filled with a brown substance; sometimes one finds a cell completely filled with this brown substance except for a clear central space which apparently had been previously occupied by the nucleus.

While in the upper brown tissue, it is not uncommon for the pollen-tube to branch two or three times (Fig. 8): however, as soon as the tubes reach the lower limit of this tissue (which is about May 12) only one branch is continued. The future growth downward through the thin-walled tissue, rich in starch, is comparatively enormously fast. By the 19th, one week later, the pollen-tube had in almost all cases reached the top of the endosperm, and in one case found, had penetrated between the neck-cells of the archegonium. In pollen-tubes at this stage, it is no longer possible to distinguish the nucleus of the pollen-tube from that of the stalk-cell. One usually finds them both close together in the lower end of the pollen-tube. They are apparently beginning to degenerate and are already considerably reduced in size and more refractive than they appeared in the earlier stages. Pl. IV, Fig. 12 shows a piece of a pollen-tube isolated from the surrounding tissue by means of the method, recommended by Belajeff, for making preparations of the pollen-tubes of *Taxus baccata*. Single ovules are laid in a mixture consisting of 2 parts of sulphuric acid and 100 parts of picric acid to which is added an equal volume of water. After being in this mixture for twenty-four hours they are carefully washed in several changes of water. The pollen-tubes may then be, in the case of *Taxus*, isolated with ease by means of needles. With *Pinus silvestris*, however, this method is seldom successful.

By May 26 the pollen-tube had usually reached the embryo-sac. In many cases fertilization was already accomplished and some divisions of the fertilized oosphere had occurred. When the pollen-tube reaches the oosphere, not only do the sexual nuclei pass into the latter, but even the two asexual nuclei also; so that in no case was either the nucleus of the pollen-tube or that of the stalk-cell observed to remain behind, and it was possible in very many cases to find them in the protoplasm of the oosphere (Figs. 13, 14). Along with these nuclei much of the protoplasm of the pollen-tube passes into the oosphere carrying with it numerous grains of starch, so that one often finds sections presenting the appearance shown in Fig. 15, where the nuclei are followed by a tail of protoplasm, or in Fig. 16, where their track is marked out by the starch-grains which have come from the pollen-tube, for before the pollen-tube reaches the oosphere, the latter contains no starch. These nuclei persist for a considerable time and are to be found in the protoplasm of the oospore after its nucleus has divided several times (Figs. 16, 17). In no case did I find the nuclei in the act of passing from the pollen-tube into the oosphere. The apex of the pollen-tube is often seen to be furnished with a deep pit, which may appear very like a perforation (Fig. 18). I am, however, fortunate to be able to quote Prof. Strasburger's opinion on this point. He was kind enough to examine several preparations, such as is figured in Fig. 18, and he regards them as showing examples of the pit first described by Hofmeister [1] and afterwards by Schacht [2], and by himself [3].

[1] Pringsheim's Jahrb. für Wiss. Bot., Bd. I, p. 71.

[2] Sitzgsber. d. Niederrh. Gesells. für Natur- und Heilkunde zu Bonn, 1864. III. Folge, Bd. I, p. 94.

[3] Befruchtung bei den Coniferen, 1869, pp. 11, 13, 14. Since the above was written, I find that G. Karsten (Cohn's Beiträge zur Biologie der Pflanzen, Bd. VI, Heft 3, p. 367) describes and figures the pollen-tube of *Gnetum Rumphianum* and of *G. ovalifolium* at the moment of fertilization, showing that it is perforated at the apex and that through this perforation its contents pass into the embryo-sac.

Immediately before fertilization radial striae can be seen extending from the nucleus of the oosphere into its surrounding protoplasm. These striae very probably precede a re-arrangement of the centrosomes of the nucleus as Guignard[1] found to be the case with the primary nucleus of the embryo-sac of *Lilium*. As a consequence of this rearrangement, the centrosomes of the female nucleus, which at first would be situated beneath the nucleus, owing to the fact that the ventral canal-cell is cut off from it shortly before (Pl. V, Fig. 19), would separate from one another and take up a position on the equator of the female nucleus. The male nucleus when it enters the oosphere is very difficult to identify; but I have observed several times similar striae round a nucleus which is in all probability the male nucleus. These striae would also indicate an adjustment of the centrosomes in its case. Accordingly, when the sexual pronuclei and their centrosomes unite, the new centrosomes formed by the fusion of the male and female centrosomes would lie in a plane perpendicular to the longitudinal axis of the archegonium. And so we would expect to find the first division of the oospore in a horizontal plane, as is in fact observed.

Only one of the two male nuclei unites with the female nucleus in fertilization, the other remains in the protoplasm of the oosphere and is hard to distinguish from the two asexual nuclei coming from the pollen-tube. Why two sexual cells are formed when one is sufficient for fertilization, is difficult to explain. In the Angiosperms, where a similar division into two similar sexual cells takes place, Prof. Strasburger[2] has succeeded in observing, in one case, an oosphere which was fertilized by the two nuclei, a fact which proves conclusively that both are truly sexual cells. Perhaps the division of the body-cell into two sexual cells came about when the branching of the pollen-tube, before described (Fig. 8), was the rule, and when two branches at least had some probability of reaching different oospheres. Belajeff has observed that the pollen-

[1] Nouv. Etudes sur la Fécondation, Ann. des Sc. Nat., Bot., 1891, p. 181.
[2] Bef. bei den Phan., p. 64.

tubes of *Taxus* also branch. In the Angiosperms too it is not uncommon for two or more pollen-tubes to be formed from one pollen-grain and to be continued for some distance [1].

Reduction of the number of Chromosomes.

It has recently been shown that, in several plants, at least, there is a reduction in the number of chromosomes in those cells which give rise to the sexual apparatus, i. e. the mother-cells of the pollen-grains have a smaller number of chromosomes than are found in the other cells of the plant, and this reduced number is preserved with great constancy in future divisions of the nuclei inclosed in the pollen-grains. Likewise, it has been shown in a number of cases that a reduced number is also present in the primary nucleus of the embryo-sac, and that this number corresponds with the number of chromosomes in the pollen [2]. Guignard, however, has shown that, in the case of the primary nucleus of the embryo-sac, all the nuclei resulting from its divisions do not preserve the reduced number of chromosomes. Thus, during the division of the primary nucleus of the embryo-sac of *Lilium Martagon*, he counted 12 chromosomes, and similarly he saw 12 in the nuclei arising from its upper half; but in the divisions of the lower half 16, 20 and 24 chromosomes were counted. In the divisions of the secondary nucleus of the embryo-sac to form the endosperm the number of the chromosomes is variable; but it is remarkable that the first division shows more chromosomes than the other cells of the plant.

E. Overton [3] also found that a similar reduction in the number of the chromosomes took place in the reproductive cells of *Ceratozamia mexicana*, *Tsuga canadensis*, *Larix decidua*, and *Ephedra helvetica*. He found in fact that, while the cells of the nucellus contained 16 chromosomes, those of the young endosperm contained but 8. From this fact Overton concludes

[1] Strasburger, Bef. bei den Phanerog. pp. 41, 44.

[2] Strasburger, Kern- und Zelltheilung, Jena, 1888, p. 51 ff., and 241 ff., and Guignard, Nouvelles Etudes sur la Féc.

[3] 'On the Reduction of the Chromosomes in the Nuclei of Plants.' Annals of Botany, March, 1893.

for Gymnosperms 'that it is in the highest degree probable that the reduction in the number of chromosomes is effected during the formation of the embryo-sac and persists through the whole female gametophyte (endosperm) including the oosphere.' The same writer also found that in the Gymnosperms, as in the Angiosperms, a similar reduction takes place in the mother-cells of the pollen and persists through the whole male gametophyte.

In this connection it became interesting to study the instances of karyokinesis exhibited in the nuclei of the endosperm and the various tissues of *Pinus*. The small size of these nuclei, however, rendered the counting of the chromosomes very difficult; nevertheless I have endeavoured, by multiplying my observations and by making careful drawings of the karyokinetic figures observed, to render my results reliable. From these drawings I found that the dividing nuclei of the cells of the nucellus, in those cases where the chromosomes could be counted, possess 16 chromosomes: Fig. 20 includes examples of the sketches made. In most cases it is best to draw those nuclei which one can view from one of the poles, as in them the chromosomes appear more widely separated from one another. The nuclei of the cells of the integument possess 16 chromosomes and perhaps rarely 24. On examining then the young endosperm, I found that in nuclei taken from its middle and lower portions there were only 8 chromosomes, Fig. 21. Thus, so far, my observations on *Pinus* agree with those of Overton on other Gymnosperms. However, in pursuing my examination, I was surprised to find that, in sections made through the endosperm about June 19, which showed the archegonium and oosphere already formed, the cells forming the wall of the archegonium, which are much larger and have larger nuclei than the surrounding cells of the endosperm, possess usually a larger number of chromosomes: the chromosomes in their nuclei are widely separated and are consequently easily counted with certainty. The prevailing number of chromosomes in these nuclei was not 8 but 12; I also found some cells with 24. Fig. 22 shows 4 cells from

the wall of an archegonium of which 3 have 12 chromosomes and one 24. In some cases too 8 were found. This led me to examine the youngest endosperms I had at my disposal, i. e. of April 24, and I found that there is in these a group of larger nuclei at the upper end of the embryo-sac which certainly, in some cases, have 12 chromosomes. At this stage the walls of the archegonium were not yet formed by cells provided with membranes. In several specimens both of the early and later stages, where nuclei with a larger number of chromosomes were seen, it was possible also to find nuclei in the lower parts of the embryo-sac which had only 8 chromosomes. Fig. 23 shows a nucleus from an endosperm of this date with 12 chromosomes. Accordingly it appears that, even in a comparatively early stage of its development, the gametophyte is composed of two distinct kinds of cells, i. e. those with nuclei containing 8 chromosomes and those whose nuclei have 12 or 24 chromosomes. To which of these kinds does the oosphere belong? As is well known the oosphere divides shortly before fertilization to give rise to the ventral canal-cell; so this would seem to be a suitable time to count the chromosomes of its nucleus. However, although I found this division very frequently, I never succeeded in counting the chromosomes of its nuclear plate, owing to the very minute quantity of chromatin present and the consequent difficulty in staining (Fig. 24). Strangely enough, however, the chromosomes of the ventral canal-cell arising from this division remain for some time separate from one another. Fig. 25 is a drawing of the cell in this state, and shows that it contains only 8 chromosomes. I counted this number in two other specimens also. From this we may conclude that as the canal-cell arises by karyokinesis from the oosphere and as the former has 8 chromosomes, so the oosphere has 8. As the chromosomes during this division could not be counted, it is not certain that the number in the oosphere is 8, more especially as Prof. Strasburger[1] counted 12 chromosomes in the nuclei of the pollen-grain.

[1] Ueb. das Verhalten d. Pollens; Hist. Beitr., IV, 1892.

Summarizing, then, the numbers of the chromosomes in the gametophyte, we have:

Nuclei of Prothallium (endosperm) . .	8.
Nuclei of walls of archegonium . .	8, 12, 24.
Nuclei of oosphere and ventral canal-cell .	8.

From these numbers we see that Overton's generalization is not applicable to *Pinus silvestris* at least. For, just as Guignard observed that in the embryo-sac of *Lilium Martagon* the lower nucleus resulting from the division of the primary nucleus of the embryo-sac has a greater number of chromosomes than the upper one, so here there is in certain nuclei of the embryo-sac a larger number than in others. It is also remarkable that neither in *Pinus* nor in *Lilium* is this increased number constant; in the former 8, 12, 24 were counted, while in the latter Guignard observed 16, 20, 24, whereas the oosphere and its sister-nuclei had only 12 chromosomes. It seems probable that in both cases the reduced number is only rigidly adhered to by all the nuclei of the gametophyte till a few cell-generations before the division which gives rise to the oosphere, and that at this point there arise two distinct cell-families, one with the reduced number to which the oosphere belongs, and the other with a larger variable number of chromosomes which, in the Lily, gives rise to the lower half of the secondary nucleus of the embryo-sac and the three antipodal cells, while in *Pinus* it gives rise to the cells forming the wall of the archegonium. In this way we may regard the synergidae, the upper 'polar nucleus' and the oosphere of Angiosperms as homologous with the endosperm and oosphere of Gymnosperms, while the lower 'polar nucleus' and antipodal cells of the former would correspond to the cells of the wall of the archegonium of the latter.

In the male gametophyte, on the other hand, we could not expect to find an increase of the number of chromosomes, as neither of the asexual nuclei divides after the mother-cell of the two generative cells is formed.

The first division of the fertilized oosphere was several times observed, but, owing to the paucity of chromatin and consequent difficulty of staining, I only once succeeded in counting the chromosomes with certainty, and I found, just as we would expect from comparison with the Angiosperms, 16 chromosomes (Fig. 26). This nuclear plate was formed in the middle of the archegonium in an oblique position. Subsequent divisions, which took place at the base of the archegonium, were also observed (Fig. 27). These were characterized by very pronounced striae radiating from the poles into the protoplasm of the oosphere. Fig. 28 represents a peculiar structure which was found in the protoplasm of the oosphere before fertilization, exactly resembling the nuclear spindle in karyokinesis, without having a trace of chromatin evident. In several cases the achromatic threads show a greater refractiveness about the middle of their length, exactly resembling the beginning of a cell-plate. The pollen-tube had only reached the top of the neck of the archegonium in the specimen from which these are figured.

Having seen that the nuclei of the nucellus had 16 chromosomes in the cases counted, while those of the integument had also sometimes 24, it seemed interesting to ascertain the number of the chromosomes in the nuclei of the primary meristem of the growing-point. Unfortunately I had no suitable material of *Pinus silvestris*, and had to use instead *Pinus Laricio* and *Picea orientalis*. In these two plants the nuclei of the primary meristem contained 16 chromosomes in each case where these could be counted. In sections below the apex the number was 16 in the cambium and 24 in the young cortex. Sufficient cases, however, were not examined to render it certain that this is a general rule.

I wish, in conclusion, to express my gratitude to Prof. Strasburger for his great kindness to me during my work in his laboratory, and to Dr. F. Noll for his assistance to me on many occasions.

EXPLANATION OF THE FIGURES IN PLATES III, IV, and V.

Illustrating Mr. Dixon's paper on the Fertilization of *Pinus silvestris*.

The figures were drawn, by the aid of a camera lucida, from preparations made from alcohol-material.

PLATE III.

Fig. 1. Two pollen-grains on nucellus; April 24. ×750.

Fig. 2. Body-cell divided into two and broken away from stalk-cell; April 24. ×750.

Fig. 3. Two male cells breaking away from stalk-cell; April 24. ×750.

Fig. 4. Two male cells entering the pollen-tube, followed by the nucleus of the stalk-cell; April 24. ×750.

Fig. 5. Similar to Fig. 4; April 24. ×750.

Fig. 6. Longitudinal section of the upper portion of the nucellus showing two pollen-tubes. The nucleus of the stalk-cell is just passing the two male cells; May 5. ×250.

Fig. 7. Portion of a pollen-tube. Nucleus of the stalk-cell has passed by the male cells; May 5. ×750.

Fig. 8. Branched pollen-tube seen in a longitudinal section of the nucellus; May 12. ×250.

Fig. 9. Longitudinal section of the upper part of the nucellus. The male cells preceded by the nucleus of the stalk-cell have come into proximity with the nucleus of the pollen-tube at the apex of the tube; May 12. ×625.

Fig. 10. Longitudinal section of the upper part of the nucellus. The annular wall of the stalk-cell in the pollen-grain is seen in optical section; May 19. ×625.

Fig. 11. Pollen-grain emptied of its contents; May 5. ×750.

PLATE IV.

Fig. 12. Portion of a pollen-tube, isolated by maceration. The two male cells are seen above, and the nuclei of the stalk-cell and of the pollen-tube are lying side by side in the apex of the tube; May 19. ×625.

Fig. 13. Pollen-tube and upper part of the archegonium shortly after fertilization; May 26. ×750.

Fig. 14. Pollen-tube and upper part of the archegonium. The larger nucleus in the cytoplasm of the oosphere is probably the male nucleus surrounded by radial striae; May 26. ×750.

Fig. 15. Apex of pollen-tube and archegonium. The trail of protoplasm from the pollen-tube is seen in the oosphere; June 6. ×250.

Fig. 16. Longitudinal section of the archegonium, showing the starch-grains from the pollen-tube in the cytoplasm of the oosphere; May 26. ×250.

Fig. 17. Longitudinal section of the archegonium after some divisions of the oospore; May 26. ×250.

Fig. 18. Apex of pollen-tube immediately after fertilization; May 26. ×625.

PLATE V.

Fig. 19. Formation of the ventral canal-cell; May 26. ×625.

Fig. 20. Instances of nuclear division from the nucellus. ×750.

Fig. 21. Nuclear division in a young endosperm; April 24. ×950.

Fig. 22. Cells from the wall of the archegonium; May 19. ×750.

Fig. 23. A dividing nucleus from the upper part of a young endosperm; April 24. ×950.

Fig. 24. Nuclear division preparatory to forming the ventral canal-cell; May 26. ×750.

Fig. 25. Ventral canal-cell. ×750.

Fig. 26. First division of the nucleus of the oospore; May 26. ×750.

Fig. 27. Nuclear divisions in the base of the archegonium; May 26. ×625.

Fig. 28. Spindles formed in the cytoplasm of the oosphere; May 26. ×750.

Studies in Hepaticae: On Pallavicinia decipiens [1], Mitten.

BY

J. BRETLAND FARMER, M.A.,

Fellow of Magdalen College, Oxford; Assistant Professor of Biology at the Royal College of Science, South Kensington.

With Plates VI and VII.

THE genus *Pallavicinia* (Gray), otherwise known as *Blytia* [2] (Endl.) or *Steetzia* (Lehm.), is one which includes rather more than twenty species, which exhibit, amongst themselves, a considerable range of variation in form. Most of them agree more or less with the type as illustrated by *P. Lyellii*, a plant not unfrequent in this country, and which consists of an elongated thallus, with a prominent mid-rib. Branching may occur dichotomously, or by ventrally-formed shoots. But specimens of *P. Lyellii* not unfrequently depart from the more usual form, in so far as the thallus-wings are often scarcely developed in the posterior region of the plant, which in this part comes thus to consist of little more than mid-rib. It is only as one passes to the more anterior portions nearer the apex, that the lateral expansions attain their full breadth. When however this feature is also accompanied by the formation of a few rapidly succeeding dichotomies in the apical region, the plant assumes an aspect quite foreign to the normal habitus of the species. It is, in these cases, not difficult

[1] This plant was described by Mitten (Journ. Linn. Soc., 1861) as *Steetzia decipiens*.

[2] In the Synopsis Hepaticarum the generic name is given as *Blyttia*; though Endlicher wrote it as in the text.

to conceive of the posterior almost wingless portion developing as a rhizome, whilst the expanded branch-system would form a 'frond.' This distinction into stipe and 'lamina' is actually met with in some species, e. g. *P. pisiformis*, where, to judge from herbarium specimens, the stipe forms a creeping stem, passing gradually into the 'lamina.' But this morphological differentiation of external form culminates in those species of which *P. decipiens* may be taken as an example.

In this plant there is a well marked creeping subterranean rhizome, from which arise expanded stalked aërial 'fronds' or dichotomous branch-systems. In luxuriant specimens, especially when dried, the general appearance strongly recalls that of some of the simpler Hymenophyllums, but in the fresh, living state, its succulent and turgid character forms a very striking and distinctive feature. It closely approximates in vegetative structure to many species of another genus of Hepatics, *Symphyogyna*, although it is readily distinguishable from them by the character of its fructification.

It is a fact worthy of mention, that within the limits of the genus *Symphyogyna* itself, much the same sort of transition from a simpler to a more complex type of vegetative structure may be traced. This latter genus has however outstripped *Pallavicinia*, in this respect, since it also exhibits considerable diversity in the *directions* of its complexity. Thus, besides exhibiting a gradual advance along lines parallel with those already indicated as occurring in *Pallavicinia*, in other groups of species the thallus becomes deeply indented, so as to become pinnatifid (*S. sinuata*), and so much so as to almost resemble one of the pseudo-foliose genera. It is however interesting to notice that on the same plant branches may arise whose margins are in no way sinuate, but resemble those of any common form in their regular outline; and on the other hand it is perhaps not improbable that in the toothed margins to be met with in some Pallavicinias, there is represented a rudimentary stage of that development which gives such a striking appearance to *Symphyogyna sinuata*.

Moreover there are a number of other genera which used to

be united either with *Pallavicinia* or with *Symphyogyna*, on account of their similarity in the vegetative structure of their gametophytes, but which are now separated owing to the important differences between their respective sexual organs or their sporophytes. In fact, similarity in the vegetative structure of the gametophyte, in many of these Hepatic genera at any rate, affords but unsafe ground on which to found any views as to natural affinities and mutual relationships. The existence of parallel developments, as above indicated, is a sufficient example of the soundness of this statement.

Pallavicinia decipiens has been described as occurring on the Horton Plains in Ceylon, and I found it abundantly in this locality, growing on the muddy banks of a stream at an elevation of about 6,200 feet. The specimens from this district, as well as others which I found on the summit of the Knuckles (6,000 ft.) further northwards, were comparatively stunted forms, though they were fruiting freely. At a lower elevation (4,500 ft.) the plant assumes a much more luxuriant form, and it is not uncommon at this elevation on the slopes of Adam's Peak, especially on the paths up from Maskeliya or Ratnapura. Here it grows in large patches and in such profusion as to take complete possession of the soil, to the exclusion of other plants.

Branching.—The plant consists, as has been already said, of an underground stem, and an aërial part consisting of fronds. The latter are formed simply as the ends of rhizome-branches, which, after growing horizontally in the earth for a time, bend upwards, and after emerging from the ground at once begin to flatten out, and branch in such a way as to give rise to the appearances of dichotomously branched fronds (Pl. VI, Fig. 1). At the outer side of the angle made by this upward curving of the underground stem, one or more rhizome-branches are given off, which penetrate the soil, and may either branch in it or at once turn upwards, and in their turn form fresh aërial fronds. But whatever the ultimate fate of the branches, they always arise exogenously throughout the plant.

In the aërial portions the branching which results in the

formation of the frond agrees with that form of dichotomy, so common in Hepatics, in which the original apex ceases to segment, and is replaced by two fresh apices arising right and left of it. This takes place repeatedly, and so the expanded branch-system or frond is formed. When an underground shoot is about to grow up out of the ground, it changes its shape, and from being circular in transverse section, it becomes elliptical, and wings appear on the two edges, which eventually spread out and form the laminae on either side of the mid-rib of the thallus.

The branching in the *subterranean* portion of the plant is very much complicated by the fact that the apical cells of lateral axes do not develop immediately they are differentiated, but usually remain dormant often for a considerable time. These 'eyes,' or resting buds, are constantly met with in sections near the apex of rhizome-branches, and it becomes a matter of considerable difficulty to tell whether the case is one of dichotomy or of monopodial lateral branching. It is certain however that if a dichotomy exists, it does not resemble the ordinary Hepatic type, for there can be no question of the replacement of one apex by two fresh ones; the original apical cell of the shoot is *not* obliterated, but continues to remain active, at any rate until its shoot issues from the soil. The view I have been led to adopt, after examining a somewhat large number of preparations, is that the branching in the underground shoots is strictly lateral. The apical cells of the lateral axes are formed at a very early period in the history of the segment cut off from the apical cell; they may even appear as the result of its first division, which cuts off an inner from an outer cell, the latter forming the new apical cell of the lateral axis. These new apical cells never, so far as I have observed them, develop at once, although they sometimes form one or two divisions, after which the large cell, whose size and relative richness in protoplasm mark it out from the surrounding ones, either remains dormant for a time, or, as is still more frequently the case, it does not develop any further at all.

What has been said will show that it is impossible to draw any conclusions as to the presence or absence of adventitious branches (in the true sense of the word) on the rhizome, but structures of this character occur occasionally on the ventral surface of the 'frond,' and commonly beneath a mid-rib near the spot where it forks. I have never seen these adventitious shoots develop, but their rudiments may not seldom be seen, especially when examining sections in which archegonia occur (cf. Pl. VI, Figs. 11–13). As however in the majority of the species of this genus, ventral shoots occur, it is a matter of some interest to meet them also in this aberrant type, although they never, under normal circumstances, pass out of a rudimentary state.

Structure of the Rhizome.—Transverse sections of the rhizome show a clearly marked strand of fibrous cells, whose walls become much thickened and exhibit a number of shallow pits on their walls, at first view resembling ordinary striation. The individual cells are very much elongated, and possess long pointed ends; when examined in sections near the apex, it is seen that the thickening commences in the cells in the middle of the strand, and extends from thence to those at the periphery. Immediately surrounding the strand are the large parenchymatous cells which constitute the chief portion of the rhizome, and which are bounded externally by an epidermis-like layer, the cells of which frequently grow out to form root-hairs (Pl. VI, Fig. 8).

The apex of an underground shoot is occupied by a single cell of peculiar form. The actual determination of its shape is beset with numerous difficulties, but after comparing sections cut in different directions one is led to the conclusion that it resembles a prism whose cross-section is that of an isosceles triangle, whilst the outer (free) and inner surfaces are parallel and they lie in planes at right angles to the long axis of the prism. This is a most unusual form for an apical cell, but the evidence is conclusive as to its occurrence in this plant. Transverse sections of the apex in good preparations always show a *triangular* cell occupying the centre, with

obvious segmentation parallel to its three sides (Pl. VI, Fig. 9): on the other hand longitudinal sections, cut in the plane of the ground, exhibit an *oblong* structure, with apparently oblong segments given off parallel to the sides and inner face (Pl. VI, Figs. 3, 4, 6). Owing to the height of the cell, several consecutive sections can easily be cut through it, both longitudinally and transversely, and the results are consonant with the above formed conclusion as to its actual form.

Sometimes however longitudinal sections exhibit an apical cell of apparently *triangular* outline, similar to that found in the foliose forms (Pl. VI, Fig. 5). But this appearance is due to the section having really traversed the large apical cell in an oblique direction, when of course this appearance would be produced. It is easy to convince oneself of the validity of this explanation by examination of serial sections.

Apart from the appearance as shown by the transverse section, it might be argued that the cell really is a wedge-shaped one, as occurs, according to Leitgeb, in many frondose Jungermanniae, e. g. *Blasia pusilla,* and the triangular shape in longitudinal section would, of course, result in such a case if the plane of section happened to be parallel with one of the triangular sides of the wedge, whereas an oblong form would appear in sections passing through planes at right angles to this. But the fact that *transverse* sections of the apex exhibit the triangular form above described, disposes at once of this view, since a wedge-shaped cell with a triangular head could not exist as such, but would be converted into something like a tetrahedron.

The apex of *Pellia epiphylla*, according to Leitgeb's description, accords somewhat with that of *Pallavicinia decipiens*, but in the former the prism is rectangular, and not triangular, in transverse section. Abscission of segments parallel to the innermost face of the cell is however a feature common to both. The form of segmentation prevailing in our plant, as will be seen by comparing Leitgeb's figures [1], differs in important characters from that exhibited by *Pallavicinia*

[1] Leitgeb, Unters. ü. d. Lebermoose, Heft III.

(*Blyttia*) *Lyellii*[1], which was especially studied by him, and this fact affords another example of the marked dissimilarity of form and structure in the vegetative organs, which may exist in even allied species, and the difference described by Leitgeb[2] as existing between *P. epiphylla* and *P. calycina* in the structure of their respective apices is another case in point.

Division in the lateral segments takes place by means of walls which first cut the segment into an inner and an outer cell, and then, in the ordinary vegetative segments, a further wall parallel to the segment-wall appears. The further divisions were not studied. The apical cells of branches are formed very early, and are delimited by the first wall which appears in the segment, cutting off the inner from the outer cell. It is the latter which becomes the young apical cell of a lateral axis.

The segments given off parallel to the inner face of the apical cell divide chiefly by longitudinal walls, and contribute to form the central strand of the rhizome, which has been described above.

When the dormant resting apical cells of lateral branches are about to become active, walls appear in them which eventually cut out a cell similar to that which has already been described for the mother-apex. But the new walls are somewhat irregular at first (Pl. VI, Fig. 10), and it must be borne in mind that the cell in its dormant condition is in form a rectangular prism, which of course is a consequence of the way in which it originates in the young segment. The new walls then have to cut out a triangular prism, but it frequently happens that this is not effected until several divisions have occurred, and it is to this fact that the somewhat irregular appearance as regards the arrangement of the cells at the new apex is to be attributed.

One result of this lateness in the development of the branches is seen in a feature which was also noticed by Leitgeb in *Pallavicinia* (*Blyttia*) *Lyellii*, and doubtless the explanation is similar in both cases. The axile strand of

[1] Loc. cit. [2] Loc. cit.

prosenchymatous pitted cells of the branch never unites with that of the mother-axis, but it ends blindly in the base of the branch. The cells in this region are short, and their cavities are large, and this is of course due to the fact that at this early period, the longitudinal divisions which cause the fibres to be so narrow in the more developed parts of the branch, were much less frequent at the time of its incipient growth, while at the same time transverse divisions were much more abundant than is subsequently the case.

A biological result of this lack of union is seen in the relative independence of the daughter-axes and the ease with which they become severed from the mother-shoot, to form fresh plants.

Structure of the Fronds.—The 'frond' is formed, as has already been stated, by rapidly succeeding dichotomy accompanied by a development of the thallus-wings, which are suppressed on the creeping portions of the plant. The apex exhibits the same structure as in the subterranean parts, though it is more difficult to determine its structure here. The actual apical cell lies at the base of a sinus, caused by the rapid growth of the segments, and it is bathed in the mucilage poured out by the hairs formed for this purpose. The fresh apices which arise as the result of the pseudo-dichotomy in the frond do not behave like those of the creeping stems; on the contrary they develop immediately, and thus the mid-ribs of the different axes are not here disconnected, but form a forked system corresponding to the branches of the frond. The edges of the lamina are toothed, and these teeth are situated as filiform hairs on a multicellular base, but they do not appear to be formed in a definite manner from the segments. The occurrence of adventitious ventral shoots on the fronds has already been alluded to. They *may* have been formed near the apex of the frond, but I do not think this is the case. Moreover, the structure of their apical cells is different from that of growing axes, since they always exhibit a *triangular* outline in longitudinal sections of the frond (Pl. VI, Fig. 12), and this probably

is due to their being in reality wedge-shaped cells. It may here be pointed out that there is not a great dissimilarity between a wedge and a prismatic cell such as has been described for the growing parts of this plant. A triangular prism may be conceived of as a broadened wedge, and the difference, so far as the plant is concerned, lies in the respective positions which such a wedge takes up in the apex. If its cleaving edge is directed inwards, and its head forms the free outer surface, then the ordinary 'wedge-shaped' apical cell arises. If on the other hand, the wedge is situated so that its cleaving edge is directed vertically, its head ventrally, whilst its triangular sides form respectively the inner and outer faces of the cell, then a form of cell arises such as is met with in the growing axes of *Pallavicinia decipiens*. Moreover it is further possible that there is a special significance attaching to the shape of the apical cells occurring on these rudimentary adventitious branches, as pointing to an earlier type from which the triangular prisms of the rest of the shoots have been derived.

Sexual Organs.—The plants, so far as my own specimens show, are strictly dioecious, and the antheridia and archegonia occur on the dorsal surface of the branches of the frond. The latter are grouped over a dichotomy, whilst the former occupy the length of one or more of the final branches of the frond, and are commonly situated on either side of the mid-rib in considerable numbers. A branch of the thallus bearing antheridia differs from the sterile ones inasmuch as it is considerably narrower, and in this feature it recalls the behaviour of the pinnae of many Ferns, where the sporophylls differ from the sterile leaves of the same plant (e. g. species of *Lomaria*).

The antheridia arise as papilliform projections of cells very near the apex, and do not offer any special features of interest in their development. They ultimately form globose stalked bodies each situated in a depression of the frond, and roofed over by a scale which arises behind them, but leaves an opening anteriorly (Pl. VII, Fig. 18), through which the spermatozoids escape.

The archegonia occur in groups upon cushion-like protuberances which arise immediately over a 'vein,' and almost always at a spot where it is just forking. Each archegonial group is enclosed in a circular involucre, whose free margin is toothed or fimbriated.

There are seldom more than two or three archegonial groups formed on a frond, though I have seen as many as seven, and they are nearly always confined to the early branchings of the frond, and never, so far as I have observed, extend to the younger dichotomies.

The archegonia in their development conform to the ordinary Hepatic type and need not therefore be dealt with here, though it may be mentioned that paraphyses, or mucilage-hairs, occur plentifully amongst them. Even before fertilization, a second sheath can be discerned within the involucre, but it is only after fertilization that this develops into the large colesula which is characteristic of the genus. This further growth however takes place immediately upon fertilization, and is the first visible sign of its having been effected. All division in it is limited to an annular zone situated at its base, just where it joins the frond, and nuclear division may be favourably studied in this region.

But besides the effect of promoting the development of the colesula, fertilization also brings about a vast increase in the number and in the growth of cells occupying the base of the archegonial group, as well as in the fertilized archegonium itself. In this way the massive calyptra is formed, within the colesula, and it carries up the numerous barren archegonia on its growing surface. As at the base of the colesula, so also in this tissue, dividing nuclei can be conveniently studied.

Nuclear Division in the Gametophyte.—The nuclei are very small, but they possess the great advantage of containing but few chromosomes, and in all cases when I have been able to estimate their numbers with certainty, there have always been present four chromosomes to each nucleus. Gentian violet and orange used as a double stain are very valuable in researches of this nature, the vividly stained

violet fibrils standing out sharply on a brownish-coloured ground.

No importance can be attached however to results in counting chromosomes, whenever they are as small as is the case in the present instance, unless they can be seen grouped at the equatorial plane, and can be regarded from the *poles* of the spindle. A study of a profile-view may be very misleading, since owing to the minuteness of the bodies, and their closely-aggregated condition, it is extremely difficult to avoid overlooking some of them, or to be in any way certain of the correctness of the results obtained. Seen from the poles, in favourable preparations, the chromosomes are seen to be in the form of loops, and to be four in number (Pl. VII, Fig. 37*a*). The loops split longitudinally in the usual manner, and often in the diaster-stage it becomes a matter of difficulty to feel sure of the actual numbers, even when seen from the pole of the spindle. As the two sets of loops are now moving along the direction of the line of vision, it is obvious that they will present their *ends* to the observer, and thus each individual loop will appear as two dots (Pl. VII, Fig. 37). As the number of the chromosomes has become doubled, previously to moving apart from the equatorial plane, it is clear that at this stage there will be sixteen dots visible at the equator, but that they will not be in the same focus. It only requires a little care however to understand the facts as they thus appear. Beyond the *number* of the chromosomes, the nuclei do not present any especially interesting features, but in the particular just mentioned they acquire considerable interest when compared with the nuclei of the sporophyte.

The Sporophyte.—After fertilization, the oospore grows into a somewhat pear-shaped body within the rapidly enlarging venter of its archegonium, and it soon divides transversely into three cells (Pl. VI, Fig. 15). The lowest cell undergoes very little further division, and contributes in but a slight manner to the structure of the embryo. The middle cell rapidly divides, both transversely and longitudinally, and forms both the stalk and the chief part of the anchor-like

base of the sporogonium. The lining superficial cells of the lower part of the stalk and swollen base are remarkable in the richness of their protoplasmic contents, and in the large size of their nuclei. They perhaps play a part, not merely passive, in the absorption of food from the gametophyte.

The capsular portion of the sporogonium originates from the uppermost cell, but as the sporogenous cells and the wall-layer are not differentiated until somewhat late, it is possible that the upper discs, into which the middle of the three first cells falls, may also contribute a share in the formation of this part of the sporogonium. Divisions in the young capsule are by no means regular, but eventually the tissue which ultimately goes to form the spores and elaters becomes clear, by the relative richness of its cells in protoplasm.

In the young stages of the development of the sporogonium, instances of nuclear division are not unfrequently to be met with, and a striking difference is at once seen between these nuclei and those, already described, of the gametophyte. In the first place the sporophytic nuclei are relatively large, but the important difference lies in the fact that there are not four, but *eight* chromosomes, also of a looped shape, to each nucleus. Even a profile-view awakens a suspicion that the chromosomes, when in the equatorial plane, are more numerous in these nuclei than in the above mentioned gametophytic nuclei, and a view from the pole of the spindle amply confirms this. Fig. 34 illustrates such a view, and it will be seen that there are, in all, thirty-two dots shown, sixteen lightly, and sixteen darkly, shaded.

As a matter of fact this nucleus is in the diaster-stage, and the daughter-chromosomes are beginning to move apart. Those marked more lightly represent the ends of the retiring loops of one nucleus, whilst those shaded darkly belong to the loops of the other one. The chromosomes form U-shaped bodies here, and hence there are in reality only eight chromosomes to each nucleus, the double number (sixteen) being due to the fact that the loops are seen in the direction of the

shanks, which thus appear as dots, and of course twice as many as the actual number of the loops.

This relation between the nuclei of the sporophyte and the gametophyte respectively, affords strong confirmation to Overton's[1] view of the meaning of the halving of the numbers of the chromosomes in the reproductive cells of Phanerogams, when contrasted with the corresponding vegetative nuclei.

As the development of the sporogonium proceeds, the wall becomes several layers of cells in thickness, and the contents of the capsule differentiate into elaters and spore-mother-cells. The former are characterized by their increasingly elongated form, whereas the spore-mother-cells finally become rounded bodies. The elaters are scattered through the sporogenous tissue, and do not form strands, such as occur in *Aneura* or in *Pellia*.

Development of the spores.—The spore-mother-cells are somewhat poor in their cytoplasm, which contains large quantities of oil. A nucleus of large size, enclosed in a special protoplasmic mass, occupies a central position in the cell. Presently the characteristic change which precedes the actual formation of spores in the Hepaticae becomes apparent. The mother-cell becomes tetrahedrally lobed, and the cell-walls, at their inner angles, grow into the cell-cavity towards the nucleus.

This body is surrounded, as has been said, by somewhat dense protoplasm which probably represents an archoplasm, and just before nuclear division it behaves in a most striking manner. Simultaneously it projects out into each lobe of the mother-cell, and thus forms a four-rayed star, with the nucleus occupying the centre. These arms, or spindles together form what I have recently, in a paper communicated to the Royal Society[2], termed a 'quadripolar spindle.' The spindle-arms or rays stain deeply with nuclear stains, and I am strongly of opinion that the substance from the *nucleolus*

[1] E. Overton, On the reduction of the Chromosomes in the Nuclei of Plants, Annals of Botany, vol. VII.

[2] Proc. Roy. Soc., vol. LIV, No. 330, 1894.

becomes diffused out into them. The objects are too small to admit of this suggestion being verified by observation, but I have already shown [1], and my results have been confirmed and extended by Zimmermann [2], that the nucleolus may break up and pass out into the cytoplasm during division in pollen-mother-cells, so there is no inherent improbability in the view that a similar state of things obtains here. The chromatic elements of the nucleus next begin to become individualized, and, so far as I have been able to determine, on studying many hundreds of preparations, they do so in an irregular manner. It is possible that the spirem-stage may occur, as it certainly does in the vegetative cells of the sporophyte, but I have never seen it. Four chromatic droplets form the first positive evidence of approaching division which I have seen *in the nucleus.* The quadripolar spindle however has been formed *before* these four chromosomes appear. In several instances I have seen the centre of the nucleus occupied by a large mass of chromatic substance, which was distinctly four-lobed, as though the four bodies, already referred to, arose by the breaking up of an original lump in this way. Fig. 30 is a camera-lucida-drawing of such a case, and I may say that I possess photographs of this actual specimen which exhibit exactly the same appearance as that shown in the drawing.

Shortly after the appearance of the four chromosomes, their arrangement becomes more regular, and their number is increased by division to eight. Their shape is not that of *loops*, as is the case with the vegetative nuclei, but that of *rods*, and it may be remarked that this difference of form is extremely common between the vegetative and reproductive nuclei of the same plant, e. g. in *Lilium Martagon.* The eight chromosomes now point off in pairs as though about to travel to the lobes of the spore-mother-cell, and I believed until recently that only two chromosomes went to each lobe. I have however since found two instances, so clear as to admit of no

[1] J. B. Farmer, On nuclear division in the Pollen-mother-cells of *Lilium Martagon*, Annals of Botany, vol. VII.

[2] Zimmermann, Beitr. z. Morph. u. Physiol. d. Pflanzenzelle, Bd. II. Heft 1.

doubt whatever, in which, just before the exit from the centre, a further doubling of the chromosomes occurs, so that in reality four of these bodies, and not two, as I previously supposed, go to form the nucleus of each spore. The whole process is very much crowded up, the four-rayed spindle persisting to the end; and even after the exodus of the chromosomes, traces of it can still be seen, converging to the original centre. Moreover, so late is the whole process, that the sculpturing which characterizes the walls of the mature-spore is well advanced before nuclear division is even incepted. In many cells, the retreating chromosomes could be traced, but there nearly always appeared to be only two: this however arises from the fact that I only saw them in profile, and not from the pole, and hence the two nearer ones covered the pair lying below. However in the young nuclei of the spores, whose walls have not as yet been formed to meet in the centre, the four chromosomes could sometimes, though with difficulty, be made out. The archoplasm, which forms the spindle, breaks asunder, and each arm contracts up along with the young nuclei, thus forming a sort of sheath around them, like that which surrounded the original mother-nucleus.

The spores become finally separate by the appearance of membranes which complete the isolation of their contents in the centre of the tetrad, an isolation which is however nearly effected by the earlier ingrowths mentioned above.

Now these features attendant on spore-formation in *Pallavicinia* are so abnormal, or at least depart so widely from our ordinary notions of cell-division, that I deferred publishing them until I should have had the opportunity of comparing it to the process as it obtains in other Hepaticae. I have examined the process in a number of other genera, and am still engaged in extending the observations, which will be brought together in a future communication. In this place I will only refer briefly to these results in so far as they help to elucidate the case of *Pallavicinia*. If the process be followed out in *Aneura multifida*, a form which is abundant in this country, essentially the same phenomena are perceptible,

but the process is, as it were, slowed down. At a certain time, and previously to any visible change in the nucleus, a quadripolar spindle is formed, each of the four rays projecting into one of the four lobes of the spore-mother-cell. At the extremity of the rays may be seen a distinct protoplasmic aggregation which represents a centrosphere, but a centrosome was not observed.

Now this quadripolar spindle is *transient* in *Aneura*, for when the chromosomes become delimited and individualized, the original quadripolar spindle breaks up and is replaced by two *independent* spindles in the equators of each of which there may be counted eleven, or possibly twelve chromosomes. These are excessively small, and it is difficult, and perhaps not very important, to determine their absolute number. Then each spindle acts independently, and the chromosomes (which here too are in the form of short rods) become doubled, and nuclear division takes place as on normal lines.

Aneura pinguis, of which I obtained a good supply when in Ceylon, also exhibits very plainly the quadripolar spindle, which diverges from the relatively large nucleus. In this plant it can be clearly seen that the nucleus, at the stages at which my specimens were fixed, is passing into the spirem-stage. I could not, owing to failure of material, obtain the stages of actual nuclear division in this plant, but the presence of such a spindle is of interest.

In *Lophocolea* I have also seen nuclear division proceeding on the same lines as I have just described for *Aneura multifida*, but I have not seen as yet anything like a quadripolar spindle. It may however ultimately prove to be there, and my failure to recognize it hitherto may be due to its transient existence—a difficulty which I had experienced already in *Aneura*—in *Lophocolea*, as in *Aneura*, and there was the same difference between the reproductive and vegetative nuclei, as regards the shapes of the chromosomes; i. e. in the former they appear as rods, in the latter as loops.

It is obvious, from what has been said, that a serial

modification from normal division to a possibly reduced, and at any rate much compressed, type of karyokinesis is traceable within the genera comprised in the Hepaticae. There are, furthermore points of theoretical interest connected with the modifications just described, but it is best to postpone the discussion of these questions until further investigations have rendered it possible to review and compare the process in a wider range of species than can be done at the present time.

EXPLANATION OF FIGURES IN PLATES VI AND VII.

Illustrating Mr. Farmer's paper on *Pallavicinia decipiens*, Mitten.

Figs. 1-37 refer to *Pallavicinia decipiens*; Figs. 38 and 39 to *Ancura multifida*.

Fig. 1. Barren plant (natural size).

Fig. 2. Fertile frond (enlarged). *a*, involucre, surrounding a group of archegonia: *b*, colesula, from which a sporogonium is issuing.

Fig. 3. Longitudinal section of apex of rhizome, in horizontal plane, with apical cell: *c*, the apical cell of lateral branch.

Fig. 4. Longitudinal section of rhizome-branch which is growing up to form a frond.

Fig. 5. Longitudinal section of rhizome, somewhat oblique, with an apparently triangular apical cell: *c*, apical cell of branch.

Fig. 6. Young branch of rhizome.

Fig. 7. An older ditto, showing the central strand.

Fig. 8. Transverse section of rhizome.

Fig. 9. Transverse section of apex of rhizome: *a*, apical cell: *c*, apical cell of branch (cut below the surface).

Figs. 10, 10 *a*. Young apices of lateral branches forming their apical cells.

Figs. 11, 12, 13. Adventitious buds, from ventral surface of frond beneath archegonial groups.

Fig. 14. Apex of a branch of the frond.

Fig. 15. Group of archegonia, one has been fertilized and contains a three-celled embryo: *i*, involucre: *p*, colesula.

Figs. 16, 17. Young embryos.

Fig. 18. Longitudinal section of antheridial branch (somewhat diagrammatic).

Fig. 19. Spore-mother-cell, with ingrowing cell-walls.

Figs. 20-29. Stages in development of spores.

Fig. 30. Spore-mother-cell with four-lobed chromatic mass.

Fig. 31. Nucleus from young sporogonium preparing for division.

Figs. 32, 33. Different views of dividing nuclei from young sporogonium.

Fig. 34. Dividing nucleus in diaster-stage, from the same tissue. Only the *ends* of the looped chromosomes are shown.

Fig. 35. Dividing nucleus in colesula.

Figs. 36, 37, 37 *a*. Dividing nuclei of cells at the base of the calyptra.

Fig. 38. Spore-mother-cell of *Aneura multifida*, with quadripolar spindle.

Fig. 39. Karyokinesis in spore-mother-cell of the same.

A Contribution to the Physiology of the Genus Cuscuta [1].

BY

GEORGE J. PEIRCE, S.B.

With Plate VIII.

INTRODUCTION.

IN the course of an investigation into the origin, development, and structure of the haustoria of certain parasitic Phanerogams, the results of which were recently published [2], a number of questions presented themselves which could not be answered from alcohol-material, the only kind then available. Some of the questions concerned the members of the genus *Cuscuta*, and required, for satisfactory study, considerable quantities of these plants alive and in all stages of development. An abundance of such material was most generously put at my disposal by Professor W. Pfeffer, of the University of Leipzig, who very kindly gave me a place in his laboratory and helped me at all stages in my work by suggestions and criticism. The plants used were *Cuscuta Epilinum*, Weihr., cultivated on *Linum usitatissimum*, L.; *C. europaea*, L., on *Urtica dioica*, L., and on various florists' varieties of *Chry-*

[1] Diss. Inaug. at the University of Leipzig, 1894.

[2] Peirce, G. J., On the Structure of the Haustoria of some Phanerogamic Parasites, Annals of Botany, vol. VII, no. XXVII, 1893.

[Annals of Botany, Vol. VIII. No. XXIX. March, 1894.]

santhemum; and *C. glomerata*, Choisy, on *Impatiens Sultani*, Hook., *I. Balsamina*, L., and *I. parviflora*, DC., &c. Of these the two larger, *C. europaea* and *C. glomerata*, proved most convenient for experimental purposes, owing to their own greater vitality, and also to the fact that their host-plants (except *Urtica*) bear confinement in a rather dry greenhouse very well. *C. glomerata*, an American species, is decidedly better for indoor study than *C. europaea*, attaining under such conditions a larger size than the latter, though differing from it in this respect only slightly out of doors; and the translucent character of its hosts, their large-celled pith and cortex (especially of *I. Balsamina*) make it an almost ideal plant for histological as well as physiological observation.

I began my experiments late in April, and continued them, without interruption, until the middle of August. Some of the material was raised from seed in the comparatively dry, at times cool, at times very hot, greenhouse which forms part of the botanical laboratory of the University of Leipzig. More of it was cultivated in the Botanic Garden: host and parasite were sown or planted together in pots in a frame, and when the hosts had become a few centimetres high, and the *Cuscuta*-seedlings had become well attached to them, the clumps of soil enclosing the roots were removed undisturbed from the pots and set into a warm, not too sunny, well-watered bed. Still more was sown with the seeds of the hosts, quite without precautions in dry and sunny, or in somewhat moist and shady, places. The rest of the material was taken by transfer (as will be described further on) from an unpotted to a potted host, and brought into the small greenhouse above referred to. Owing to the unusual dryness of the season, accompanied in May and June by longer or shorter periods of abnormal coolness, the plants out of doors were not so luxuriant, and did not so long vegetate, as in more favourable seasons, but came sooner and more compactly into bloom. As will be shown further on, these differences from the usual conditions have made clearer some interesting facts which otherwise might have escaped my notice.

From the time of Palm[1] and Von Mohl[2], who studied certain species of *Cuscuta* in the course of their investigations on climbing plants in general, these interesting parasites have been so much studied and written about that, for the sake of a tolerably complete presentation of the new observations which I have to communicate, I must repeat much that is generally known. The student of the plants of this genus is especially indebted to the papers of Ludwig Koch[3], and to the second of these I shall have frequent occasion to refer.

In this paper I shall consider *first* the formation of the haustoria, the conditions necessary for this and for their full development, the general relations of the parasite to its environment; and *second* the penetration of the haustoria into the host.

I. The Formation of Haustoria.

1. *Germination and early growth.*

If the seeds of one of the three species of *Cuscuta* above mentioned be sown on moist earth, they gradually absorb sufficient water to double their size, and, in eight or ten days, germinate, pushing through the integuments of the seeds roots which are snowy white in colour. Such a root, short, thick, more or less spindle-shaped, devoid of a cap, and with short, though rather numerous hairs (their length varies directly with the amount of moisture), is very feebly geotropic and penetrates the subjacent soil for only one or two millimetres, if at all. Instead of the cells of its central cylinder differentiating into vascular bundles, which are at first radial and separate, later, through secondary thickening, becoming collateral and confluent, the central cylinder consists of no lignified cells at all; the walls remain thin, and, though the

[1] Palm, L. H., Ueber das Winden der Pflanzen, Stuttgart, 1827.

[2] Mohl, H. v., Ueber den Bau und das Winden der Ranken und Schlingpflanzen, Tübingen, 1827.

[3] Koch, L., Untersuchungen über die Entwickelung der Cuscuteen, Hanstein's Botanische Abhandlungen, Bd. II, Heft 3, 1874; Die Klee- und Flachseide, Heidelberg, 1880.

cells become quite long, they retain throughout their brief existence the appearance of young procambium-cells, being filled with granular protoplasm enclosing large nuclei. This core of thin-walled elongated cells, less like a vascular structure than the central cylinder of the larger Mosses (e. g. *Polytrichum*), is the only indication of the differentiated root-structure which these plants must have possessed before they became parasitic. The cortical cells are approximately cubical, thin-walled, containing abundant protoplasm and large nuclei. Indeed it is a noteworthy, as well as an easily observed, fact that the nuclei of all the living cells of a *Cuscuta* are larger than those of the corresponding cells of its host. The epidermal cells and the root-hairs are slightly cutinized.

The root having penetrated the soil for a short distance, or having grown sufficiently over the surface to give some support, the now rapidly elongating stem, having grown for some distance out of the seed, bends at a point near its union with the root and thus rears its tip, still enclosed within the seed-coats and imbedded in the endosperm from which it draws its nourishment, till it becomes nearly or quite erect. The young stem is yellow or almost white, at any rate not green, and so lacking in chlorophyll that assimilation must be very slight, if at this stage of the plant's history it occurs at all. Frank[1] cites in his text-book an investigation carried on in his laboratory by Temme[2], according to which chlorophyll is present, not merely in quantity sufficient for spectroscopic determination, but also for the evolution of oxygen, as proved by experiments with fuming phosphorus. Such investigations were, however, carried on with older plants, and, though necessarily implying the presence in the seedling of plastids capable of developing into chloroplastids, scarcely yield results which would justify our assuming that the seedling obtains food from any other source than the endosperm in which its tip is imbedded. (As to the amount of chlorophyll

[1] Frank, A. B., Lehrbuch der Botanik, Leipzig, 1892, Bd. I, p. 556.

[2] Temme, F., Berichte der deutschen botanischen Gesellschaft, 1883, p. 485. Also in Landwirthschaftliche Jahrbücher, 1884, Bd. XIII.

in older plants I shall have more to say further on.) The stem being now erect or nearly so, and continuing to grow in length, nutates in wider and wider circles or ellipses in search of some suitable object around which to twine. As first pointed out by Von Mohl [1], the seedling will not twine indiscriminately about any object whose size, form, and position one might suppose to be appropriate, if not directly to nourish, at least to hold it up in its efforts to reach a nutrient support, namely some living plant. Von Mohl tested this by pieces of wire and thin rods of fir-wood kept for three days in contact with the seedling. The plant refused to twine about them, though, when brought into contact with a plant of Nettle, it wound about it within nine hours. Koch [2] and others have confirmed this important observation, but without being able to account for the fact. Koch [3] says—'Der junge Schmarotzer besitzt somit in Bezug auf den von ihm zu befallenden Gegenstand eine gewisse Wahlfähigkeit, deren physiologischer Nutzen nicht zu verkennen ist.'

Through the integuments of the seed the embryo receives from the moist soil, or damp substratum of any sort on which the seed may rest, sufficient water to cause it to germinate, other conditions of temperature, &c., being favourable. The spindle-shaped and but feebly-developed root continues to secure moisture through its hairs from the substratum, and thus provides the necessary amount of water for the solution and transportation of the food-substances stored in the endosperm. The root is, however, a short-lived structure, beginning to die generally within seven days after its appearance, and hence it can supply only a small quantity of water in all. Under favourable conditions, primarily a damp substratum and a moist atmosphere, the root doubtless supplies enough water completely to convert the reserve food-materials in the endosperm into transportable and available solutions; but if, owing to the surrounding air being dry, the transpiration of

[1] Mohl, H. v., loc. cit.
[2] Koch, L., Hanstein's Botanische Abhandlungen, Bd. II, Heft 3, p. 110, 1874.
[3] Koch, L., Die Klee- und Flachsseide, Heidelberg, 1880, p. 17.

the seedling be greatly increased, and the neither thick-walled nor strongly-cutinized epidermal cells of the stem be unable to counterbalance the loss by their own absorption, the growth of the seedling will be greatly lessened in speed and amount, and thereby its chances of finding a suitable support will be diminished. Any dry object with which it comes into contact also draws water from it. Hence it is evident that, in this respect at least, it is disadvantageous to the plant to wind about dry and innutritious supports. The length to which the seedling can grow is proportional to the amount of water which it can absorb and retain, that is, to its turgescence. Seeds sown on sunny dry beds in the garden germinate slowly, the roots of the seedlings die soon, the stems attain a length of only a few centimetres, usually not more than five, unless a host be quickly found. If, on the contrary, the bed be a moist and shady one, or still better, if the seeds be sown on damp earth in a pot covered by a bell-glass, the seedlings can attain surprising lengths. Not only is the total amount of growth greater, but the length of the living part of the seedling is also greater. From the dying root most of the nutritive materials are removed to younger parts. Thus fed, the tip of the stem can continue to grow, and thus the area explored by it in its circumnutation is increased. Not only does the dying root yield its substance for the nutrition of the tip of the stem, but the stem dies also from below upwards, and from it too the nutritive substances are transferred to the youngest parts. Owing to the ability of the growing part to extract food from the oldest and least useful parts, the seedling is capable of a slow locomotion. The advantage of this is quite obvious, for in this way the seedling can approach a suitable plant which was at first beyond reach, and finally can lay hold on it.

If the root be not already dead before the seedling has found a host about which it can twine, it quickly dies when the youngest part of the stem begins to wind. The direction of winding in every case which has come under my observation was in the direction of circumnutation, namely the reverse

of the hands of a watch. The stem of the seedling. like the stem of the older plant, as I shall show later, is sensitive to contact-irritation for only a short distance from the tip and only in the growing part, and the irritability is greatest near the middle of the growing region, diminishing rapidly in both directions. If, therefore, the stem be brought by its nutation into contact with a host so that a non-irritable or only slightly irritable part be applied to the host, either no twining will take place, or only a loose steep spiral will be formed, until the irritable part touches. Then the *Cuscuta* rapidly forms one or more close spirals, the direction of the curved part of the stem being as nearly horizontal as possible. As I shall show later, the object of these close spirals is two-fold : they bring many more points of the stem into intimate contact with the host ; and they hold the stem, which would otherwise be pushed away from it by its own growth and by the growth of the haustoria, closely applied to the host. When such close spirals are made, haustorial formation is induced by the intimate contact. The origin, structure, and development of the haustoria were described and figured in detail in my previous paper[1]. Suffice it to say here that the haustoria originate, like typical roots, deep in the cortex, break through the overlying cortical and epidermal tissues, penetrate into the host and, attaching themselves to the vascular bundles, draw from them through tracheids and sieve-tubes the various solutions which they contain. Exactly how the haustoria make their way into the host was one of the questions which I set myself to answer, and of this I shall speak at length in the second part of this paper.

One or more turns in a short close spiral having been made about the host, the root, unless already dead, and the stem below the first point of contact, die quickly, yielding their substance as nourishment to the youngest parts of the seedling. The root and stem remain for only a short time as an empty, dry, shrivelled filament which the wind quickly snaps and blows away. The nourishment obtained from them

[1] Loc. cit.

enables the young plant to increase considerably in diameter where it has twined (an increase in size like that well known in tendrils[1]), to form haustoria, to develop the tabular epidermal cells overlying the nascent haustoria into long papillate cells, such as I have already described[2], and whose special functions I shall presently discuss. During the process of winding about the host, growth in length becomes slower, and finally almost, if not altogether, ceases, while the plant is increasing in diameter, forming haustoria, and sending them into the host. The haustoria having penetrated by the means which I shall describe later on, and having united their phloëm- and xylem-elements with the phloëm- and xylem-elements of the vascular bundles of the host, as I have described in the preceding paper[3], the young *Cuscuta* now receives an abundant supply of food. Much of this food it accumulates in solid form before it begins to grow again in length, as the abundance of starch-grains in the cortex indicates. When considerable quantities of reserve material have been thus accumulated, the plant, now entirely parasitic, grows for a time very rapidly, not only the main stem increasing in length but one or more buds, found generally two or three together in the axils of the small scales which are the only leaves that the plant has, rapidly develop into branches whose number, length, and thickness are proportional to the number of haustoria which the *Cuscuta* has been able to form, and upon the nutritiveness of the host.

The age and size of the host at the point where it is attacked greatly influence the parasite. If the host be of considerable diameter, two centimetres for example, the seedling will usually fail to effect a single turn about it: or if it be one-and-a-half centimetres, the parasite can make only one turn, and thus will not clasp firmly enough to enable many haustoria to penetrate; hence the supply of food obtained will not be

[1] Darwin, C., Movements and Habits of Climbing Plants.
[2] Peirce, G. J., loc. cit.
[3] Id., loc. cit., p. 295.

favourably proportioned to the effort made to secure it, and the *Cuscuta* often succumbs under such conditions. If, however, the host be one centimetre or less in diameter, the seedling, unless exhausted by the length to which it has already been obliged to grow in order to reach a host, can make a number of turns, will clasp the host tightly, will form many haustoria in consequence of the extensive area of intimate contact, and will therefore be the more likely to obtain abundant nourishment. The maximum diameter of a stem or branch of a living plant around which a seedling can twine varies with the species of *Cuscuta*, the larger species, and naturally also the larger seedlings of the same species, being able effectually to embrace larger hosts than the smaller ones. One and a half centimetre is the mean maximum for *C. Epilinum*, and two centimetres for *C. europaea* and *C. glomerata*. I am unable to give the minimum. I have seen, however, seedlings winding successfully about stems of one millimetre in diameter. Since older plants, even of the larger and therefore, for mechanical reasons, less conformable species, find no difficulty in winding tightly about, and even forming incipient haustoria against, silk and cotton threads of a mean diameter of one-third of a millimetre, there is little reason for supposing that, other conditions being favourable, a seedling could not wind closely about and send haustoria into a host of no larger diameter.

The part of the host attacked need not be cylindrical in form, as the frequent observation of seedlings, as well as older plants, which have applied themselves to the lamina of various sorts of leaves, proves. In such a case the very sharp turn which the stem of the parasite is able to make allows it to apply itself to both the upper and the under surface of the leaf. The whole curve thus made is an ellipse, one of whose diameters may be very short, the other proportionally very long. Such an ellipse made by a seedling around a leaf had a long diameter of one centimetre and a half, a short diameter of two millimetres. A similar curve made by an older branch of *C. glomerata* had as respective diameters a line nearly

three centimetres long and another one millimetre long. By the great range of curves which these parasites can make, they are enabled to conform themselves to the most variously-shaped parts of their hosts.

Another quality of the host must now be considered, however. If the first plant about which a seedling of *C. glomerata* lays its coils be an *Impatiens* of one of the three species mentioned above, the haustoria will penetrate easily and find abundant nourishment. But if the seedling fastens itself upon some other plant its chances may not be so good. If the plant be a hard and comparatively innutritious Grass, or a tolerably woody herb or shrub, the haustoria will penetrate more slowly and will secure less nourishment, and hence the parasite will not so soon be able to develop its stem and branches, or so abundantly. Thus its chances of quickly finding a more nutritious host are lessened. The success of the *Cuscuta*, its dangerousness as a parasite, depend in the first place upon the number of seeds each plant forms, upon the distribution of these in places where many herbaceous plants grow close together, where the ground is moist, or where the abundance of larger herbs form a covering under which a moist atmosphere is confined, and hence where transpiration is not excessive.

2. *Conditions necessary for the formation and development of haustoria.*

The quickly-growing main stem and branches enable the young parasite to reach out in many directions, to lay hold upon the branches of its host, upon other plants, and indeed upon all objects which can mechanically support it, be they wet or dry, smooth or rough, living or dead. When the plant has secured a suitable host and has entered upon the period of healthy activity which an abundant supply of food ensures, it is in the most suitable condition for experiment.

After a period of short close winding about a host, a period

of but slow growth in length, of considerable increase in thickness, of active development of haustoria, there follows a period of long loose winding, of rapid growth in length, of slight increase in thickness, and of no development of haustoria. The biological advantage of this alternation in mode of growth is great and evident. During the period of rapid growth in length the parasite greatly increases the area, often also the number of plants, from which it may draw food. During the period of slow growth in length it fixes itself at many points in this area and develops the haustoria which are to supply it with food. The means by which these alternations in mode of growth are accomplished must now be considered.

As stated above, though the seedling will not twine about dry and dead organic supports, or rods of glass and metal, yet the young plant, feeding itself from the host upon which it has fastened, will do so. Hence, in the following experiments, rods of wood, glass, glass covered with filter-paper, wires, strings, and threads, as well as plants, can be used, provided none be too large in diameter. It makes also no difference, as will be shown presently, whether the rods be wetted throughout the course of the experiments or remain dry.

If now one of the objects named be brought into contact with one of the young and rapidly developing lateral branches, or with the main stem when it begins rapidly to grow after forming haustoria, though the contact be carefully maintained, either by frequently moving the rod in correspondence with the change in direction or the increase in length of branch or stem, or by fastening the branch or stem to the rod in any way (best by a narrow ribbon of gummed paper), the *Cuscuta* will make no close turns about the support. If it follows the support, as the more or less erect main stem is likely to do, and as the more nearly horizontal branches are not likely to do, only a long, steep, loose spiral will be formed. After a time, however, the growth decreases in rapidity. If now a contact be made about three centimetres from the tip, the stem or branch will bend sharply and, in the course of fifteen

hours, make two or three short turns closely around the vertical rod. Within the next fifteen hours more such turns may be made, and it will be evident from the swellings on the side of the *Cuscuta* next to the support, that haustoria have begun to form. If the contact between the *Cuscuta* and the vertical rod be made at a point only about one centimetre from the tip of such a more slowly growing part, there will be little or no effect. If the point of contact be too far from the tip, more than six or seven centimetres for *C. glomerata*, there will also be little or no effect; if far enough back, absolutely no effect.

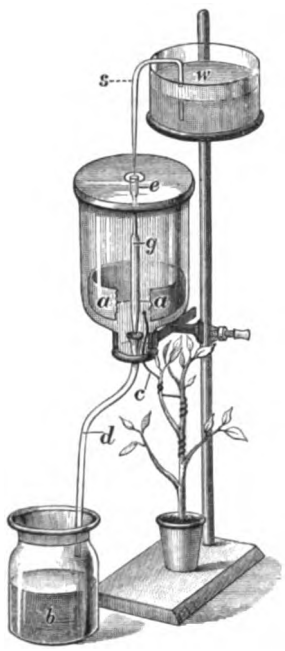
The parallel of such a condition of affairs is exactly furnished by tendrils, for example of *Passiflora gracilis*, Link. A wooden, metallic, or glass rod placed in contact with a young, short, and rapidly-growing tendril, whether the point of contact be near the tip or the base, or in the middle, will produce no effect. When, however, the tendril ceases to grow so rapidly, having attained a length of several centimetres, on such a rod being brought into contact with it at a point about three centimetres from the tip and against the side toward the direction of nutation (the concave side), twining will take place. If the contact be made at or very near the tip, or in the region nearer the base which has ceased altogether to grow, there will be no effect. Every one knows that the tendrils are sensitive to irritation by contact, and it is the general opinion that the stem of *Cuscuta* is so also, but so far as I am aware no sufficient proof of this has yet been adduced, though since Von Mohl first made the statement in 1827 many have studied the plants of this genus in various ways. In a very suggestive paper by Pfeffer[1], one finds an account of a series of interesting experiments on tendrils, in which it is clearly shown that fluids (unless they are poisonous or contain solid particles in suspension) produce no irritation by contact. Pfeffer found also that gelatine, if kept wet, also produced no irritation by contact, but, when allowed to dry,

[1] Pfeffer, W., Zur Kenntniss der Kontaktreize, Untersuch. aus dem Bot. Institut zu Tübingen, Bd. I, p. 483 et seq., 1885.

at once induced the usual turning of the tendrils. At Professor Pfeffer's suggestion I undertook to test the irritability of *Cuscuta* by means of gelatine. If the *Cuscuta* wound about a rod coated with wet gelatine, forming short close turns, there must be some reason other than contact-irritation (perhaps a chemical one) for their formation, for *Cuscuta* does not of itself and unstimulated make such close spirals ; though old tendrils which have not succeeded in finding a support normally do. If, on the other hand, the *Cuscuta* made only long, steep turns, if any, we should have positive evidence in support of the idea of contact-irritation.

According to the paper just cited, it makes no difference in its effect on tendrils whether the gelatine be simply soaked in water till it will absorb no more, or whether it be dissolved in proportions of eight per cent. or less in water. For reasons of mechanical convenience I preferred to use concentrated gelatine. I coated, as smoothly as possible, a glass rod of two or three millimetres diameter and twenty centimetres length, for a distance of fifteen centimetres, with gelatine to which only enough water had been added to make it rather soft when heated over a water-bath. In order to allow for its absorbing more water and swelling later, the coat of gelatine was made not more than two millimetres thick at this stage. Around one end, the end of the rod not coated, I wound several layers of filter-paper, enclosed this in stiffer paper, and tied it fast together, thus making a cup, out of the bottom of which, when the rod was held vertical, water could flow in a thin sheet over the whole rod of gelatine. Into a chamber made of a bell-jar held mouth upwards on a retort-stand (as shown in the annexed woodcut), covered by a glass plate pierced in the centre by a hole one and a half centimetre in diameter, and kept moist by a thick band of wet filter-paper (*a*) laid inside, I introduced a branch of *Cuscuta Epilinum* (*c*). Through the hole in the glass plate above I passed the gelatine-coated glass rod (*g*), fastening it at the top and resting its lower end in the mouth of a tube (*d*) which ran to a catch-bottle (*b*) below. From a reservoir (*w*) above

a constant supply of water was conducted by a fine siphon (*s*) into the paper cup (*e*) at the upper and uncoated end of the gelatined rod. From this cup the water flowed slowly and uniformly over all parts of the gelatine and was conducted away by the drainage-tube (*d*) to the catch-bottle (*b*). The hole at the bottom of the moist-chamber was closed by cotton wadding and the hole in the glass cover was nearly closed by a split cork. In the course of its circumnutation the *Cuscuta* came into contact with the rod, the nutation was delayed by the rigid obstacle, but the stem did not twine. Later, however, contact with the gelatined rod having been repeatedly broken and renewed in the course of the nutation of the stem, the plant began to climb up the rod, making long, steep turns about it. These processes lasted eight days. On the ninth day, the stem, having reached the upper and uncoated part of the rod, at once made short, close turns about the glass and began to form haustoria. Control experiments with uncoated rods of glass and wood, wetted and dry, of rods coated with gelatine but not wet, in similar moist chambers, showed that contact always induced the formation of close turns and of haustoria. Hence it is evident that it was not the water which inhibited close winding, but merely the non-irritant nature of wet gelatine.



These experiments repeated with plants of all three species invariably gave the same results. We see, therefore, that the formation of close turns is always dependent upon contact-irritation; that *Cuscuta* is, as others have asserted, irritable to

contact. Previous authors have pointed out, but without proving the fundamental fact of its irritability, that *Cuscuta* in its two modes of twining alternately resembles twining-stems and tendrils. It is generally admitted now, despite the efforts of Kohl[1] to prove the contrary, that climbing-plants climb by the combined action of nutation and geotropism, and that most climbing-plants are not irritable to contact. (*Lophospermum scandens* is one of the exceptions, its stems being slightly and its petioles considerably irritable.) When irritation is not produced, either through the absence of all contact with irritable parts, or through the contact of a non-irritant such as wet gelatine, the plant grows and climbs only as other climbers do, making long, steep turns about the support. Like tendrils, the stem is not sensitive in regions of no growth in length, or in regions and at times of most rapid growth, but is sensitive at times and in regions of moderate growth.

It is known that in the case of tendrils the effect of contact is not immediate, nor is it necessary that the contact should be permanent to produce winding. If momentary contact be made with very sensitive tendrils, for example those of *Passiflora gracilis*, Link, and *Sicyos angulatus*, L., decided bending toward the side on which the contact was made takes place More slowly, if the contact be not renewed, the tendril returns to its former position. The process of twining is therefore *induced*, a short period of contact inducing a comparatively slight and temporary bend, a longer one having stronger and more permanent effects, till finally a point is reached when withdrawal of the irritant object causes no return to the primary position of the organ, the induction having produced permanent effects. With tendrils less sensitive than those just mentioned, such striking effects are not produced, yet the principle remains the same. Now experiment shows that when the contact of an irritant object with the irritable part of the stem of a *Cuscuta* produces certain effects, these effects are not immediate but are *induced*; that

[1] Kohl, F. G., Beitrag zur Kenntniss des Windens der Pflanzen. Pringsheim's Jahrbucher für wissenschaftliche Botanik, Bd. XV, 1884, p. 327, &c.

if the contact be not long continued the effects, though plainly evident, are only temporary; and that contact with an irritant object need not be permanent to produce permanent effects. But *Cuscuta* is by no means so sensitive as the most sensitive tendrils. Negative geotropism plays a much more important rôle than in the case of tendrils; for repeated experiments show that the parasite will not twine about horizontal branches or rods, even though they be rough and thereby strongly irritant. If one complete turn be made about a vertical rod and the plant be evidently ready to make more, it will not continue to do so if the support be laid horizontally; but on the other hand it will only partly, if at all, undo the turn which it has already made. Also, if two or more close turns be made around a vertical rod, the formation of haustoria will not be prevented or delayed by placing the rod horizontally.

It is now evident that the short close turns of the *Cuscuta* about its host, or about any other support of suitable diameter, are induced by contact-irritation. It is also plain, from the observations of many authors, that haustoria are normally formed in nature only on such close turns. But is it necessary for the health of a branch that it form such close turns? And are such close turns necessary for the formation of haustoria? Both questions can be answered in the negative, as the two following experiments show.

A strong horizontal branch of *C. glomerata*, springing from the axil of a bract borne on a stem just above a region where many strong haustoria had penetrated a very nutritious branch of *Impatiens Sultani*, was allowed to grow horizontally without contact with anything, until it had reached a length of fifteen centimetres or more; then it was supported at a point far enough behind the tip to be no longer irritable. It continued to grow horizontally, and supports were applied at suitable distances. Finally at the end of three weeks, having sent out numerous branches (which I cut off lest, in the crowded greenhouse, they should by accident come into contact with some other plant, and so vitiate the results of the experiment), the main branch, large, healthy, in normal con-

dition so far as I could see, for its whole length, and still growing, had attained the surprising length of one metre! This experiment shows not only that close turns are unnecessary to the healthy nourishment and perfect development of a branch, but also that the nourishment secured by the haustoria of one part can be transported over great distances to those parts which are not fed by haustoria of their own. In this latter respect it makes little or no difference whether a horizontal or an erect branch be used; for a branch allowed to twine on a long vertical glass rod, from which it can of course draw no nourishment though it may make close turns about it and begin the formation of haustoria (which soon abort, however), will attain a very considerable length at the same time that its healthy appearance is preserved.

To show whether or not close turns are necessary to the formation of haustoria, the following experiment was undertaken. A branch of *C. glomerata*, in suitable condition to wind closely and to form haustoria if conditions favoured, was enclosed between the upper faces of two leaflets of a *Phaseolus* still attached to the petiole, whose lower end dipped into a bottle of water. The leaflets were kept so closely applied against the *Cuscuta* between two plates of glass of convenient size (e.g. 8 cm. long and 4 cm. broad) that it could make no curves except in the plane parallel with the surface of the leaves and glass. The glass plates were held firmly together by a stout paper band around each end. In order to prevent their crushing the leaflets and the *Cuscuta* which were pressed between them, a strip of glass, two millimetres thick, was placed between them at each end. In this way a constant contact, accompanied by considerable but not dangerous pressure, was maintained for three days. That the branch was perfectly healthy was shown by its continuing to grow in quite normal fashion. At the end of three days the apparatus was taken apart, and it was found that the parasite had made no curves, even in the plane parallel with the glass plates, but that it had formed haustoria which had entered the leaf-tissue. It is, therefore, plain that close windings are not of them-

selves necessary to the formation of haustoria. They are simply a means, and the easiest and most effective one, of bringing a considerable area of the stem or branch of the *Cuscuta* into intimate contact with the host, and of maintaining this contact. If intimate contact be formed and maintained over a considerable area in any other way, which is rarely the case in nature, the haustoria will be formed without the usual preliminary of winding.

It has been asserted by L. Koch[1] that the stems and branches of these parasites can, and sometimes do, twine closely about a host in the opposite direction to their nutation, and then form haustoria as usual on the side in contact with the host. I have not had the good fortune to see such plants. Experiments show, however, that *Cuscuta* is not more irritable on one side than on another. In the experiment just described, where a branch was enclosed between two leaflets, the contact and pressure were alike on both sides. Haustoria were formed on both sides penetrating the two leaflets equally well, the haustoria generally alternating in sets of three on the two sides; but some haustoria were directly opposite one another.

If a young branch growing quite free be marked with a straight longitudinal series of dots for a distance of five centimetres from the tip, it will presently become evident that, both in free growth and in the formation of close turns after contact with a host, a decided torsion of the stem takes place, and that the line of haustorial development does not bear any definite relation to this line of dots. From the absence of bilaterality in the stem, there is no reason why, when the close turns have once been made in the direction opposite to the nutation, the haustoria should not develop. To overcome the tendency to nutate in one direction, and to bend sharply and for a time continuously in the opposite direction, might require very strong irritation; or it might be that some plants of this genus (as in a few others[2]) nutate

[1] Loc. cit., 1880, p. 18.

[2] Compare Darwin's Climbing Plants, about *Scyphanthus elegans*, &c.

alternately in one, then in the other direction, twining according to the varying direction of nutation. Such alternation I have not seen. There is the further possibility that in some the tendency to nutate in a given direction is weaker than in most plants, and that therefore the irritation need not be unusually strong to cause winding in the opposite direction. Koch says it is not common for such turnings to be made. A very large majority undoubtedly twine in the direction of nutation, in the reverse direction to the hands of a watch. There is no more reason, however, why some plants should not twine in the opposite direction constantly, or in both directions alternately, than that there should not be left-handed or ambidextrous men and women.

I have said above that the object of the close turns is to produce and to maintain an intimate contact between the parasite and its host for the purpose of developing haustoria. I have shown too that the process of twining closely is one which is *induced* by contact, following it after a longer or shorter interval. It may now be asked if the formation of haustoria is also induced by contact-irritation, and will be continued even after the contact has ceased to exist? To answer this question a number of experiments were undertaken.

If a branch of *Cuscuta* be brought into contact with a host and, after having made two or two and a half close turns about it and being about to form more, be gently removed from it and held from below in the same general direction, the branch will, like a tendril, continue to twine for a while. During the time necessary for the formation of two turns about the host, the contact will have been so protracted as to ensure the permanence of at least the lower one of these turns, but the time is too short (being about ten hours for *C. glomerata*) for any external evidence of haustorial formation to become visible. In the course of the next twenty-four hours, the usual swellings on the concave side of the curved branch will become evident, and these will continue for some time to increase in size. They are produced by the formation

of haustoria deep in the cortex, which push out the overlying cortical and epidermal cells. No change in the form of the epidermal cells takes place, though they must multiply in order to maintain an unbroken covering for the stem. After the swellings have ceased to grow, sections of them show that the haustoria attained a greater or less development in proportion as the part of the stem was in longer or briefer contact with the host; but in no case where the contact was not permanent was there a complete development.

That haustoria never begin to form without contact has been repeatedly shown by placing branches which are ready to form haustoria, whenever the conditions become favourable, in various nutrient solutions. Haustoria never form in such circumstances. If, however, a rod of suitable size be brought into contact with the submerged branch, feeble twining will take place and sometimes haustoria will be begun; but the process of twining and, as a consequence, the formation of haustoria are hindered by the fluid. The hindrance is doubtless mainly of one sort, that which reduces the irritating effect of the rod. This result resembles in kind, though not in degree, those obtained by similar experiments with sensitive tendrils[1]; but owing to their greater sensitiveness to the contact of solid bodies, the fluid, be it water or anything else not poisonous, does not so greatly reduce the amount of twining. Evidently contact is absolutely necessary for the beginning of haustoria, and it is plain too that the formation of haustoria is an induced process, just as the winding of tendrils and the formation of short, close turns are induced processes.

The swellings just mentioned as being produced by the outward pressure of the growing, but soon aborting, haustoria, differ in shape from those formed when the parasite remains at those points in contact with a host-plant. These swellings, the results of the arrested growth of haustoria, are conical in form and are covered by flat and somewhat elongated

[1] Pfeffer, W., Zur Kenntniss der Kontaktreize, Untersuch. aus dem Bot. Institut zu Tübingen, Bd. I, p. 483 et seq.

epidermal cells exactly like those on other parts. The swelling produced by the growth of a constantly-developing haustorium under a point of continued contact with a host, is broader and not so high, and is surrounded at a little distance by a ring-like elevation of about half its height. Longitudinal sections show, as I have already described and figured [1], that the central elevation is formed by the pressure of the subjacent growing haustorium, and is covered by thin-walled, papillate epidermal cells of very considerable length and abundant protoplasmic contents; that surrounding this are a few rows of shorter, but still papillate, epidermal cells of similar character; that beyond these are several rows of long, papillate epidermal cells, these alone forming the elevated ring surrounding the central protuberance; and that beyond this the epidermal cells remain typical in form and contents. We see plainly that not only is the formation of close spirals and of haustoria a result of contact-irritation, but that the changes in the epidermal cells are also due to this cause. As will presently be shown, however, the complete development of the haustoria and a complete modification of the overlying and immediately adjacent epidermal cells is not the result of contact only.

Ludwig Koch [2] has clearly described that, when the parasite has made one or two close turns about a branch of its host and does not continue winding about this, but makes at once a similar spiral about the petiole of a leaf, which springs at that point from the stem of the host, that part of the *Cuscuta* between the last point of contact with the stem and the first point of contact with the leaf will, like the adjacent regions, bear haustoria. But the haustoria in this intermediate region will not develop far, and we shall see instead the conical swellings which Koch denotes 'sterile Haustorien.' He ascribes their formation, like that of normal active haustoria, to irritation; but he fails to make clear whether he believes them to be the result of irritation by contact at the points where they appear, or whether they are induced to form by the contact

[1] Loc. cit., p. 295. [2] Loc. cit., 1880, p. 50.

of the stem with the host on either side. To put the question directly—Is each haustorium induced by contact immediately over its point of origin, or can haustoria be induced to form between two points by contact at these two points only? The question may readily be answered by marking the growing and twining stem with Indian ink at the last points of contact with the host, and bringing against it, at a distance of one centimetre or less, either another host or a rod of suitable size. When the *Cuscuta* has made one or two close turns about the support, it will be noticed that the dots which were over the last points of contact with the host have now been brought by growth into the region intervening between the host and the new support, and that when haustoria appear there they form at or behind those points, not in front of them and towards the tip, and somewhat earlier than those on the part of the parasite which is in contact with the new support. If they were induced between the points of contact, no contact having ever existed in the region of their origin, they should appear at the same time as or later than those against the support. Their earlier appearance and the change of position of the dots make it quite evident that they are the results of contact at the points where they originate and that their abortion is due to the contact being only temporary. Each haustorium is, therefore, the result of irritation produced by contact at the place where it forms.

I said above that contact alone, though sufficient to induce the formation of haustoria and to cause some changes in the overlying epidermal cells if continued long enough, is not sufficient to secure the full development of haustoria or to produce extended changes in the epidermal cells. It has often been observed that when a branch of a *Cuscuta* has wound itself in a close spiral about a rod of glass or wood that, though the haustoria are formed, as shown by the swellings on the concave side of the stem, and also by sections, they do not develop very far. Sections show further that, though the epidermal cells at the points of contact and overlying the nascent haustoria do elongate slightly, no such

marked difference between them and those adjacent is to be seen, as has just been briefly described as existing when the parasite is in similar contact with a host. That it is not the dryness of such rods of wood, glass, paper, or whatever the material may be, which stops the development may easily be shown. If water be made to flow gently and constantly over such rods, the epidermal cells differentiate little if any more, and the haustoria do not develop any more, than if the rods remain dry. If, after haustorial formation has been strongly induced by a wet or dry rod, the rod be withdrawn and the branch be enclosed in a moist chamber made of a piece of large glass tubing, sand being carefully poured into the tube and around the branch to a height just above the last turn, and wetted with distilled water, both ends of the tube being closed by corks, no more development of haustoria takes place than has already been described as occurring when the contact is only temporary. This experiment shows plainly that it is not the hardness of the rods which causes the epidermal cells to remain only slightly differentiated and the haustoria abortive, for the haustoria could easily penetrate between the comparatively loose particles of sand, and surely the sand would cause sufficient irritation, if contact were all that is necessary to cause a full development of these two structures. The sand used was first treated with strong hydrochloric acid to remove all calcium salts, washed on a filter with distilled water till the filtrate gave no acid reaction, then heated for a quarter of an hour over a Bunsen flame to carbonize all organic matters not decomposed by the acid, and finally cooled and poured into the glass tube, which had previously been washed with strong alcohol and then with distilled water. Of course the cork which is used to close the lower end of the glass tube must be cut in halves, and a groove, large enough to enclose the branch of *Cuscuta* without pressing it closely, be made between the two pieces.

Thus we see that the continued contact of an easily penetrable substance is not sufficient to induce full development of the haustoria and of the overlying epidermal cells; nor are

moisture and contact combined sufficient. One naturally inquires if the nourishing character of the host has anything to do with the rapid and full development of these structures. To answer this question is easy. If, after having induced haustoria by contact with a rod of some sort, taking care that the contact be only long enough to induce the formation of haustoria and not long enough for the innutritiousness of the rod to have any repressive effect on them, one inserts the branch into a glass tube as above described, closing the tube at the bottom with a split and grooved cork, but sealing the hole by a few drops of cocoa-butter or a little gardener's wax, and pours into it a decoction of the usual host-plant, one sees that the haustoria develop little more, and that the epidermal cells are only slightly longer than when they are pressing against a rod of glass or wood. Hence it is plain that contact lasting until the formation of haustoria has been induced, and followed by a supply of nourishment in solution, are not sufficient to produce the effects which are evident when contact with a host is uninterrupted.

It must be admitted, however, that there are two quite unnatural elements in this experiment, namely: first, the superabundance of food immediately available if the plant can absorb it, and second, that this food is equally abundant on all sides. To eliminate both of these errors I induced haustoria to form on a healthy branch of *C. europaea* by contact with a wooden rod, and then substituted for the rod a stick of elder-pith, of about the same size, which had been soaked for some time in a hot decoction of *Impatiens Balsamina* which, as sections showed, had penetrated for at least a millimetre from the surface. I enclosed the branch thus wound around the elder-pith in a glass tube as before, keeping the air inside moist by means of wet filter-paper, and closing both ends by corks. In the course of nine days the *Cuscuta* had continued to wind closely, making in all five complete turns about the pith. Along the inner surface of these turns were as many haustoria as would normally be formed in an equal distance, and gentle pulling showed that

the parasite was by some means closely fastened to the pith. Cross-sections through the haustoria and the pith showed several interesting features. The epidermal cells were quite as long, papillate, and thin-walled as if they had developed in contact with a host-plant; their protoplasmic contents were only slightly less abundant and granular than in normal conditions; and they had penetrated the second, in some cases the third, layer of parenchyma-cells. (Of the mode of penetration I shall have occasion to speak in Part II of this paper.) The haustoria underlying these well-developed epidermal cells had advanced very considerably in their development, and were evidently pushing themselves against and through the adjacent cortical cells, though more slowly than normal. Their structure was as complete as that of young haustoria about to penetrate a host but still imbedded in their cortical matrix. That they were not farther developed, had not penetrated the overlying cortex and entered the stick of pith is not surprising; for the pith, though impregnated to a distance of a millimetre or more from the surface with a decoction of a plant, was nevertheless a comparatively innutritious substance. The decoction at first contained somewhat more nutritive matter than would at once be available in a host, but it was not constantly renewed as is the case in the living host, and it was likely to be very largely consumed by the epidermal cells, and also by the Bacteria and Fungi which are so difficult to exclude when it is not possible completely to sterilize everything used in an experiment. Yet the almost complete development of the epidermal cells, and the very considerable development of the haustoria plainly show, when taken in connexion with the results of the other experiments just described, that neither contact alone, nor nourishment alone after a period of contact, is sufficient for the complete development of these two associated structures. Both continued contact and abundant nutrition from without are necessary for full development.

I have previously pointed out that food taken into the parasite in one region can be transferred to another region at

a considerable distance which, because it lacks haustoria, is receiving no nourishment from outside. The distance to which food can be transported is dependent, of course, upon the nutritiveness of the host and the health of the parasite. One might suppose that, were the parasite strong and well fed, it would supply to a region in which haustoria had begun to form, all the nourishment necessary for their complete development, and also for the development of the overlying epidermal cells. Such does not seem to be the case, as the preceding experiments show. There is a manifest biological advantage in this; for any support which cannot furnish nourishment sufficient for the development of these two structures would also be innutritious even when the haustoria had penetrated it. The parasite tests the nutritiveness of the support by the cells most closely in contact with it. If these develop, the nourishment which they receive and pass on stimulates the associated structures to develop also. If they do not develop because of the lack of nourishment from without, there is no stimulus and no growth of the associated structures; plainly an economy of material and of energy.

How comparatively unimportant to the part forming haustoria the food which it receives from other parts of its own body is, can be plainly demonstrated in the following way. Koch[1] pointed out that pieces of the younger parts of the parasite, if cut off, behave like the seedlings; they nutate, they make close turns about a host when they come into contact with one, they form haustoria. He does not say that such 'cuttings,' if one may apply a gardener's term to them, resemble the seedlings in that they too refuse to twine about other than nutritious supports, and thus differ from their condition when still attached to the parent plant. This is, however, not the case; here the resemblance ends. These cuttings, just as if they were still attached to and receiving nourishment from the parent plant, will twine closely about sticks of wood as well as about a host, and will also begin the formation of haustoria.

[1] Loc. cit., 1880.

If one cuts off branches of *C. glomerata*, for example, about six centimetres behind the tip, that is, cuts off the growing region, which naturally would not be likely to contain a large surplus of food, and fastens these cuttings by little ribbons of gummed paper upon the stems and erect branches of a suitable *Impatiens*, they soon begin to make close turns, and to develop haustoria which penetrate the host in the same length of time that they would consume were they still attached to the parent plant. To accomplish this large amount of growth they must have received food from outside, namely from the host about which they have twined. This food can at first have been absorbed only by the papillate epidermal cells, for these alone were in contact with the host. As has been shown before, these epidermal cells not merely come into and remain in contact with the epidermal cells of the host, but they penetrate these and therefore can absorb from the subjacent cortical cells which are richer in nutrient substances. Until the haustoria have themselves entered the host, these epidermal cells absorb all the food which the parasite receives from without. They are therefore, in a physiological sense, *pre-haustoria*, though in a morphological sense they are quite distinct from haustoria.

Although these cuttings have made close coils, have formed haustoria, have sent these into the host, as quickly as if they had remained attached to the parent plant, yet certain differences are plainly to be seen between them and others which have performed the same operations while remaining unsevered from the parent. In both the cuttings and in the still attached tips, growth in length nearly, if not entirely, ceases when the formation of close coils begins; but in the attached tips a marked increase in thickness follows the close winding, while no such evident growth in diameter takes place in cuttings, though a slight increase in thickness is sometimes observable. Owing to this growth in thickness of the attached tips and the consequent increase in the rigidity of the coils, the haustoria, in entering the host, are unable to push the parasite away, but all the pressure which they exert by their

growth is expended in penetrating the host. The haustoria formed by the cuttings, on the other hand, not having in the comparatively slender curved stems a rigid base against which to press and thus to make all the force produced by their growth tell in the penetration of the host, push the parasite away from the host, thus wasting force and increasing the distance through which they must grow in order to reach the conducting tissues of the foster-plant.

Still another difference between these two is also evident, namely, in their colour. The colour of the unsevered branches resembles that of the rest of the plant, varying from a straw-yellow to a decided orange, according to the nourishment and illumination which they receive. Ill-nourished and therefore thin plants are pale, no matter how well illuminated ; but the better the illumination which well-nourished plants receive, the more intense becomes the colour. The colouring-matter is situated in small chromoplastids, orange in hue, which the cells of the central cylinder contain in much larger numbers than those of the peripheral layers. With the ordinary microscope no chlorophyll-granules can be distinguished, though the presence of chlorophyll, as previously stated, has been shown by Temme by micro-spectroscopic methods. In and about the flower-clusters of all the species of *Cuscuta* which have come under my observation there is always an amount of chlorophyll sufficient to be recognized by the microscope, and often these parts are decidedly green.

Turning now to the cuttings, one sees that, though they may have been deep orange in colour when cut, the colour has begun to change before twenty-four hours have expired, and within forty-eight hours they have become distinctly green. *C. europaea* changes more slowly than the other two species, yet this plant too becomes green within two days after cutting. Longitudinal sections show plainly that many chlorophyll-granules of very considerable size have been developed, and that their distribution is the reverse of that of the chromoplastids above mentioned, since they are much more abundant in the cortical parenchyma than in the central cylinder. Their

localization in the more superficial and hence better illuminated cell-layers, rather than in the central and comparatively dark tissues, confirms the opinion that they are functional. I have not attempted any chemical experiments to determine the amount of oxygen evolved. It is to be noticed further that if, instead of cutting off only about six centimetres of the tip of each branch, the cutting be ten or more centimetres long, there is less development of chlorophyll and more growth in thickness of those parts which have coiled closely around the host; and that, as the root and the lower parts of the stem of the seedling yielded the nutrient matters which they contained to the younger upper parts and died, so the lower parts of the cuttings decrease in size, shrivel, and die. Evidently their substance is taken away and consumed by the younger coiling parts. But as the preceding experiments show, no matter how much nourishment the part which is forming haustoria can secure from the other parts of the parasite, this is sufficient only for the growth in thickness which normally accompanies the formation of close turns around the host, and to forestall the necessity of developing chlorophyll-granules; it is not sufficient for the full development of the epidermal prehaustoria and of the haustoria proper.

The development of chlorophyll in these parasites is always a consequence of insufficient food, as the following experiments indicate. I raised three sets of *C. Epilinum* on the common Flax under various conditions. One set was raised in a suitable bed out of doors, the seeds of host and parasite being sown together. The Flax-seeds germinated first, and the young plants were growing vigorously when attacked by the seedlings of the *Cuscuta*. From the same lots of seeds I sowed still more simultaneously in pots of moist earth in a dry and rather cool greenhouse. After the seedlings of Flax and Dodder were well started, I brought half the pots into the laboratory, setting them on a window-sill. The other half I left in the greenhouse. The air of the laboratory, though somewhat warmer than that of the greenhouse, was

much dryer, and of course the illumination which these plants received came mainly from the side. Those plants which remained in the greenhouse throve fairly well, both hosts and parasites, but not so well as those which had been sown at the same time and in similar soil out of doors, while those in the laboratory were plainly unhealthy. Since the hosts did not flourish, the parasites necessarily suffered with them. There were in these three sets of plants three colours: those growing out of doors on healthy, thriving Flax-plants were not only large and strong in appearance, but they were also of the typical pale orange colour; those in the greenhouse were, like their hosts, smaller in size and evidently weaker, and their colour was pale yellow tinged with green; those in the laboratory were the smallest and weakest, and had a decided green colour.

If one cuts off the tips (say eight centimetres long) of healthy branches of a *Cuscuta* and puts them with their cut ends, some in water, and others in a decoction of the usual host (for convenience I used *C. glomerata* and *C. europaea* and their hosts, *Impatiens* and *Chrysanthemum*), cutting off under the surface of the two liquids about one centimetre from the end of each in order to ensure as perfect conduction of the liquids into the branches as possible, one will notice that within eighteen hours the colour has changed, becoming less deep orange; within forty-eight hours they have a plainly green hue; and still later both sets of cuttings will have become nearly as intensely green as the leaves of their usual foster-plants. Those cuttings kept in glasses of water merely, grow somewhat in length, little if any in thickness, but cease within three days to grow at all. Those in the decoction take on the green colour not quite so rapidly, though within a few hours of the others, grow somewhat in thickness, considerably in length, but they too presently cease to grow, and become as deep green as the others. Finally all die. From the water, which was from the general supply of the city (Leipzig), and even from the decoction, only small quantities of organic substances could be absorbed by the cuttings, and in self-

defence they were forced to provide some means of elaborating organic substance for themselves.

It is interesting to note also in this connexion that when plants of *Cuscuta* have, either in nature or in cultivation, fastened upon innutritious or deleterious hosts, they become green in colour just as the cuttings above described. Let branches of *C. Epilinum*, still attached to the mother-plant until they have developed numerous haustoria in the new host, be brought into contact with the stems of some *Euphorbia* of much the same diameter as the stems of Flax. When the haustoria have developed well, say in five or more days after the contact was made, sever the branches from the parent stem. Even before the branches are cut off one can see a change of colour, the orange being paler and perhaps tinged with green; but soon after they have been cut off they become quite green, and remain so as long as the parasite lives. The Dodder does not, however, flourish on a *Euphorbia*, and after growing weakly for a time, becoming thinner and thinner, it finally dies, and this generally before it has been able to bloom. That the green colour is not altogether, or indeed largely, due to severing the branches from their parent stem, is shown by the fact that branches still attached, which have struck their haustoria into plants of *Euphorbia*, are always weaker than those which have attacked other plants, and also differ from them in colour. That the former do not take on the same deep shade of green as those which are prevented by abscission from drawing any food from the parent is only what we should expect, knowing to how great distances and how abundantly the food secured by more fortunate and well-nourished parts is sent to others less well off. If, instead of letting a branch of Dodder wind about a *Euphorbia*, a *Linum* be used as the new host, abscission of the branch from its parent after the haustoria have well penetrated, causes absolutely no change in colour, for the new host supplies food suitable in quality and quantity. The point of the experiment with *Euphorbia* as host lies not all in the abscission of the branches, but in the development of chlorophyll because

the cuttings do not receive suitable or sufficient food from the plant which they have attacked. That this is really the case is shown also by a continuation of the previously described experiment of putting cuttings of *C. glomerata* on suitable branches of an *Impatiens*. The cuttings become green while they are coiling around and sending haustoria into the host. The green colour remains intense for a few days, then it begins to fade, the new parts are all yellowish, and finally the new plant, with the exception sometimes of the first close coils made, is as yellow or orange in hue as the plant from which it was cut. In this case the lack of suitable food is only temporary, and when it has ceased to exist, the *Cuscuta* no longer needs to be as nearly self-dependent as possible, no longer forms chlorophyll, but returns to its absolutely or almost absolutely parasitic condition. Such, however, is not the case when the branches of *C. Epilinum* are put upon a *Euphorbia*. Although haustoria are formed in numbers adequate to draw from the host food sufficient in quantity, yet the quality of the food, whether owing to the poisonous matters which *Euphorbia* is well known to contain, or owing to less evident causes, is unsuited to the parasite. It never returns to the healthy yellow or orange hue which marks it as a parasitic plant.

When the entrance of haustoria into a stem about which a *Cuscuta* has formed close coils is delayed by thick opposing cuticle, strong sclerenchyma, or intense silicification of the peripheral cells of the host, there takes place in the branch a more or less evident change in colour according as the haustoria attain their goal more or less slowly. Such is the case when branches of *Cuscuta* are brought into contact with leaves of *Aloë*, and with the stems of *Juncus* and *Equisetum*. As might be expected, the parasite does not flourish on these plants, for the difficulty of penetrating them is not set off by more abundant or more nourishing food than that more easily obtainable from other plants. On the contrary, the secretions of the *Aloë* seem to be decidedly poisonous, the haustoria not developing well after they have finally reached the fleshy

interior of the leaves. In *Juncus* and *Equisetum* the haustoria so rarely succeed in uniting with the vascular bundles that from these plants but a small amount of food can be obtained.

The fact that, when occasion demands, the *Cuscuta* can develop a very considerable quantity of chlorophyll which, in the appearance of the plastids which contain it, in their position in the tissues, and in their abundance, justifies the belief that it is a highly functional element of the plant, is most interesting confirmation of the hypothesis that this genus of plants has become parasitic within comparatively recent times and after having attained a fairly complex development such as the other and non-parasitic members of the Convolvulaceae possess. I have as yet had no opportunity of pursuing the interesting questions thus presented, but I hope to be able to do so later.

3. *General relations of Cuscuta to its environment.*

There remain to be discussed one or two general questions concerning the relations of *Cuscuta* to its environment, in view of the conditions necessary for the formation and development of haustoria, before I pass to a consideration of how the haustoria enter the tissues of the host, and attach themselves to the vascular bundles.

As already pointed out, the roots of *Cuscuta* are very feebly geotropic organs. The stems, on the other hand, are strongly negatively geotropic; for though they may be ready to make close turns about a support, provided the necessary conditions be complied with, yet if rough and thereby irritant rods of suitable size be held horizontally against them no twining will take place. The negative geotropism of the stems is stronger than their irritability. It is to be noticed, however, that when close twining has taken place around a vertical support, and haustoria have been induced by this means, changing the support to a horizontal position does not prevent the haustoria from forming, or delay them in their development. Geotro-

pism prevents the formation of haustoria only in so far as it prevents the formation of the close spirals, on whose concave surfaces they are normally formed. The biological advantages to the plant in this arrangement are plain and important. Unless it is able to reach the top, or the periphery in general, of its more or less spreading host, its neither large nor conspicuously coloured flowers will fail to attract the attention of those insects by whose visits they are cross-fertilized. Yet if, after having coiled about and having been stimulated to form haustoria against a nutritious support, the accidental bringing of this support to a horizontal position were to stop the formation and development of haustoria, much would be lost and nothing gained. For, by developing the haustoria already induced, the parasite can secure the food obtainable from an otherwise valueless host, and thus the sooner be in condition to seek a better support.

It might be supposed that, if the effect of geotropism were neutralized by revolving both host and parasite horizontally by means of a clinostat, contact would then be sufficient to induce winding about a support placed in any direction. In order to determine whether such is the case or not, I put at different times on the clinostats of Pfeffer and of Wortmann healthy plants of *C. Epilinum*, *C. europaea*, and *C. glomerata*, growing respectively on *Linum*, *Chrysanthemum*, and *Impatiens* (*Sultani* and *parviflora*), which had been growing in pots in the greenhouse long enough to have become thoroughly acclimated, revolving them around a horizontal axis for periods varying from one to five days. In no case did twining take place, however carefully the contacts were maintained. The parasites grew in length in perfectly normal fashion. The absence of irritability to contact was the most noticeable difference from their usual state. Furthermore, a plant taken off the clinostat after having been revolved horizontally and continuously for two or three days around its long axis, was not at once sensitive to contact-irritation. The period of insensibility varies in proportion to the length of time the plant has been upon the clinostat. A plant of *C. europaea*

which had been on the clinostat for seventy-two hours did not begin to form close spirals around a support until twenty-four hours after it had been restored to the vertical position; then, however, it twined and developed haustoria in the usual time.

If parasite and host be laid horizontally, the parasite will bend upwards as quickly and as sharply as the host, although at the time it may be in contact with a horizontal rod.

As shown so clearly by Wortmann[1], geotropism is such an important factor in the climbing-power of plants that we should not greatly wonder that *Cuscuta* did not wind about horizontal rods either when it was itself erect or horizontal; for, from the fact that it is only at times that the parasite winds about its support in a way different from that of an unirritable climbing-plant, in other words is irritable only at times, we see how important and how constant the effect of geotropism on its growth must be. But it is surprising that the neutralization of geotropism by revolving the plant horizontally round its long axis should at the same time obliterate its sensitiveness to contact. The effect of horizontal revolution on these plants is like the effect of an anæsthetic on animals. Sensitiveness is in both cases destroyed temporarily; in both cases growth can take place as usual throughout the period of insensibility; and after the removal of the cause of the insensibility time must elapse before the organism completely regains all its usual powers.

Though the sensitiveness to contact is destroyed when the effect of geotropism is neutralized by revolving parasite and host horizontally, yet the sensitiveness to light becomes then clearly apparent. Either the heliotropism already existing is merely made evident when the effect of geotropism is removed, or the removal of geotropism as a factor affecting the behaviour of the plant causes it to be more sensitive to light. However this may be, by controlling the direction of illumination one can cause the tips of the parasite, as it is being revolved horizontally with its host on the clinostat, to bend in any

[1] Wortmann, J., Theorie des Windens, Botanische Zeitung, 1886.

direction desired. The stem is positively heliotropic. Exposure to light from one direction for only six hours will cause the tips to point almost straight toward the source of light. If, however, the plant be simply laid horizontal, and then be illuminated from the side only, that is, so that the effect of heliotropism would be to cause the plant to grow in the horizontal plane, it will be noticed that the tips point almost vertically upwards, plainly showing how much stronger the effect of geotropism is than that of heliotropism, so much stronger indeed that most authors have hitherto agreed in saying that *Cuscuta* is not heliotropic at all. That it is somewhat heliotropic is proved by the marked effect of light on it when geotropism is excluded.

Light has little effect on the formation of haustoria, and none on the close twining of the plant which usually precedes their formation. If a branch of *C. glomerata* in contact with a wooden rod be enclosed in a dark chamber, the branch will continue to twine as rapidly and for as long a time as usual, and the formation of haustoria will be begun within the usual length of time after firm contact has been established. If anything, the branch will twine rather faster in the dark than in the light, and its haustoria will also become evident somewhat earlier; for this plant is no exception to the general rule that growth is faster at night (that is, in darkness) than during the day. Plainly the absence of light is no hindrance to the formation of haustoria; but is light a hindrance? They arise within the concave half of the close spiral formed about a host, and this is the darker half, since more light necessarily falls on the outer and convex surface of the coils. If we cause the formation of haustoria between two leaves held together face to face by two plates of glass, as already described on p. 69, we can determine the effect of light on haustorial formation by illuminating the two sides equally and unequally. When the two leaves are equally illuminated, the numbers of haustoria on the two sides of the *Cuscuta* are approximately equal. When one side of the stem receives more light than the other, the haustoria are somewhat

more numerous on the less brightly-lighted side. That there would be a great difference we ought not to expect, for the difference in the illumination of the inner and outer sides of a close-coiled spiral is also not great.

We thus see that, despite the general opinion to the contrary, *Cuscuta* is sensitive to light to a slight extent. Whether it is more sensitive to light (that is, more heliotropic) when it is green I cannot say; nor would a positive answer to this question be of great value in any way, for it is quite evident from what I have said that the green colour is formed only when the *Cuscuta* is under unfavourable conditions of nourishment. It is well known that other plants almost or entirely devoid of chlorophyll are sensitive to light and are positively heliotropic, and hence it can by no means be inferred that the comparative insensibility of this plant is due to its lack of chlorophyll. It is evident that great sensitiveness to light might frequently interfere with and counterbalance the effect of contact-irritation, and so render the plant unable to secure the necessary food. We must associate this comparative insensibility to light with its mode of attacking and spreading itself over a host, not with the absence of chlorophyll, which is merely a result of the plant's securing an abundance of already elaborated food. The lack of chlorophyll is a degeneration in consequence of parasitism (as seen in many other parasites); the neutrality of the plant to light is one of the minor aids to its accomplishing the necessary close windings about a host.

That these plants, especially seedlings, are strongly influenced by moisture I have already pointed out; but that their stems are positively hydrotropic I have been quite unable to see. If they were strongly hydrotropic they would in this way at times defeat their own ends; for it is possible to conceive that contact-irritation and hydrotropic attraction, working on two sides of the plant and drawing it in opposite directions, might neutralize each other. But hydrotropism, if at all affecting the plant as a positive force, does so only to a slight degree. When geotropism is still exercising its strong

effect, which, far from being opposed to contact-irritation, is in most instances supplementary to it, heliotropism and hydrotropism play a very small part in the economy of these parasites. The two forces which affect the plant most strongly when it is in normal condition are geotropism, which is such an important factor in all climbing plants[1], and contact-irritation, upon which all tendril-bearing plants depend to a greater or less extent for the accomplishment of the purpose for which the tendrils were designed.

Since *Cuscuta* is an annual herb, it, like other such plants, passes through the purely vegetative stage with considerable rapidity. Plants of *Cuscuta* which are cultivated for experimental purposes in a greenhouse attain their maximum of vegetative development somewhat earlier than when cultivated out of doors; but all the species (about ten in number) which have come under my notice, whether growing in a greenhouse or out of doors, were in full flower by the end of July. Up to a certain stage in the growth of a plant, all the buds which form in the axils of the scale-like bracts, the only traces of leaves which these parasites produce, develop into branches which in turn bear only vegetative buds. Presently some of the buds develop into flower-clusters. From this time on more and more buds develop into flowers, until finally few if any give rise to branches. Most of my plants of *C. glomerata* ceased to form new vegetative branches before the end of July, all their energy being devoted to flowering and setting seed. The plants of *C. europaea*, on the other hand, did form a few branches (very few in proportion to the size of the plants) until mid-October, but during the time of maximum flowering it too formed no new vegetative branches. I suspect that the greater hardiness of *Chrysanthemum* as compared with *Impatiens* has a not inconsiderable effect on its parasite, enabling it to vegetate longer than that on *Impatiens*.

The flowers of the three species discussed in this paper are sessile and in dense clusters. Manifestly when the plant is in full flower, there being no new vegetative branches, it is no

[1] Wortmann, J., Theorie des Windens, Botanische Zeitung, 1886.

longer irritable. The formation of haustoria, which depends upon irritability, which is itself dependent upon growth, is a process that takes place only during the period of active vegetation, or, in the case of *C. europaea*, after the period of maximum blooming is past and a second period of less active vegetation begins. From the time that active vegetative growth ceases and reproductive growth begins, all experimental study of the phenomena of the former must of necessity cease. I attempted by cutting off some of the young branches which were still being formed at the beginning of August on the plants in the Botanic Garden, and setting these as before described upon their appropriate hosts, to secure plants which would for a time at least continue to vegetate. All such efforts were futile. As soon as the cuttings had sent a sufficient number of haustoria (generally two sets were enough) into the hosts to secure an abundance of food, the buds already formed, and whatever new ones had in the meantime been formed, developed into flowers. Similar cuttings made in mid-October and set on Chrysanthemums growing in the greenhouse did not develop more than a few vegetative branches, though having an abundance of flowers.

Chlorophyll may be plainly recognized in certain regions of the plants, at the reproductive stage, mainly in and about the flowers, as others have before stated; but it is not abundant enough more than feebly to supplement the nutrition secured from the host.

In the majority of the plants which I have observed, the largest and finest flower-clusters were situated in or very near regions on which haustoria abounded, that is, on the close spirals. The fewer clusters on the intermediate regions were markedly smaller in themselves, and the flowers which composed them did not attain the size common in others. The advantages of being near the immediate sources of food-supply are so evident that it is unnecessary to enumerate them: but there is a mechanical advantage also in the comparatively heavy flower-clusters and the still heavier clusters of fruits being borne on the close rather than on the loose,

steep spirals; for the number of turns within a short distance and the considerably larger diameter of the stem, as well as its being firmly attached to the host by the haustoria, ensure a degree of stability and permanence unattainable in other parts. After the haustoria formed on the close spirals have penetrated, that part of the stem loosely twined in a steep spiral around the host which intervenes between two adjacent groups of haustoria, acts merely as a conductor of food from one part to another. It is no longer of mechanical advantage to the plant, and it therefore soon ceases to grow. Indeed, so slight and so unimportant does the physiological work of conduction eventually become, when no flowers are borne on the intermediate portions of the stem, that they sometimes atrophy and entirely disappear, leaving isolated the short, close spirals bearing clusters of flowers. It is plain, therefore, that the alternating regions of sensitiveness and insensitiveness which are so important in their almost totally distinct ways during the period of active vegetation, continue to be distinct in habits and in importance in the reproductive stage. The unirritable parts were of use in distributing the parasite over as much of its host as possible, and often also in reaching new hosts from which food might at the same time be drawn: the irritable parts fixed the parasite at many points in the wide area won by the rapidly-growing insensitive parts, and at these points developed the haustoria. As repeatedly proved by the experiment of simply severing or of entirely removing the stem between two such regions of haustorial formation, after the haustoria have well penetrated into the host, its further use, even at such early stages in the plant's history, is comparatively trifling. That even in nature the removal of these intermediate segments sometimes takes place, shows how independent of each other the haustorial segments are. One can, by dividing a plant in each of these intermediate regions, produce a number of individuals whose yield of seeds will be as good in quality and quantity as that of undivided plants, provided of course all other conditions are equal. Indeed, it is also possible during the latter part of

the vegetative and throughout the reproductive stage to divide the larger groups of haustoria, which sometimes bear more than one cluster of flowers, without doing any apparent damage. This too sometimes takes place in nature. In Plate VIII, Fig. 1 is shown a petiole of *Solanum jasminoides*, Paxt., around which a *Cuscuta* has twined with many close coils. Only under the four clusters of flowers is any part of the stem to be seen; the rest, above, below, and even in this close haustoria-bearing spiral, has atrophied and finally disappeared, leaving but slight traces. Manifestly these little clusters, each supplied with food by the very small number of haustoria beneath it, have now become individual plants. They remind us of the large and apparently isolated flowers of *Brugmansia* and *Rafflesia*, but we know[1] that the great flowers of *Brugmansia* (and it is the same in *Rafflesia*) are connected with one another by strands of thin-walled merismatic cells running through the cambium of their host. There is nothing, however, either on the surface or in the tissues of the host, which connects these flower-clusters of *Cuscuta*; they are absolutely isolated finally, though of course perfectly and evidently connected at an earlier stage. It is in part owing to its ability to bear, without serious if any injury, division into many small parts, and to its even itself so dividing at times, that the Dodder is such a difficult parasite to exterminate when once it has entered a field.

In most instances the anatomical effects of this parasite on its hosts are not marked. It generally robs them so rapidly and so completely that they sooner or later succumb without having combated its attacks by the formation of any new structures, and even without having shown any renewed growth as a consequence of the intruder's presence. In the petiole figured in Plate VIII, Fig. 1, however, we see a general enlargement of the whole structure and decided

[1] See Graf zu Solms-Laubach, Die Entwickelung der Blüthe bei *Brugmansia Zippelii*, Botanische Zeitung, 1876; also Die Rafflesiaceae, Engler und Prantl's Die natürlichen Pflanzenfamilien, Lieferung, 35, 1889. G. J. Peirce, loc. cit., p. 318 et seq.

enlargements in the zones where haustoria are most abundant. A section of the petiole at a point about midway between two of the flower-clusters which it bears, that is, at the line *a–b* in Fig. 1, shows (Fig. 2) that, under the single-layered epidermis (*e*) and the two or three layers of collenchyma, come many layers of cortical parenchyma-cells (*c*). Around three sides of the petiole this cortical parenchyma abuts upon the proportionally narrow phloëm-portion (*b*) of the vascular tissues. The phloëm consists mainly of sieve-tubes and bast-parenchyma, only small and scattered groups of bast-fibres being found. Separated from the phloëm by a single-layered cambium is the broad xylem (*x*), composed of rather small ducts and, in much larger proportion, of thick-walled wood-fibres. At one side, bordering upon the phloëm, is the small bundle (*l*) which runs to one of the leaflets. The vascular ring of the petiole, enclosing the pith (*p*) in which are a mass of sclerenchyma-cells (*s'*) and small scattered groups of sclerenchyma-fibres in the region marked (*s*), is incomplete on the upper side of the petiole, and here the cortical merges directly into the pith-parenchyma. An aborted haustorium is shown at *H*. The haustorium aborted probably because of the absence in this region of vascular tissues with which it could unite. After its abortion, and after the atrophy and fall of the parasitic stem which gave rise to it, this atrophy being hastened, though, I think, scarcely caused by the abortion of the haustorium, it became covered over by the epidermis of the petiole. Such aborted and partly or entirely covered haustoria are the only remaining traces of the former presence of a continuous stem wound around the petiole, and these are few in number.

If one now compares this section with the one shown in Plate VIII, Fig. 3, which is at the line *c–d* in Fig. 1, one sees at once that the larger size of the petiole at this point is due to the increase in the number of layers of parenchyma-cells in the cortex (*c*), to the formation in it of two sets (*s'*, *s'*) of sclerenchyma-masses, to the greater width of the band of wood (*x*), in which the proportion of fibres to ducts is even greater

than before, and to the presence of the large haustorium (*H*) whose phloëm- and xylem-elements have united with those of the petiole. On the upper side, the region *p*, which in the section represented in Fig. 2 was composed of ordinary spheroidal parenchyma-cells, has been the seat of considerable changes. The cells composing it have been compressed laterally into elongated forms, their long axes being at right angles to the surface, by the bending round of the incomplete vascular ring which was divided and forced apart by the intruding haustorial wedge (*H*). These cells, elongated by pressure, were then divided by cross-walls at right angles with their long axes and parallel to the direction of the compressing force. Finally a considerable thickening and lignification of the walls of all of these cells, and of some adjacent cells which had not been so much compressed and had not divided, took place. Thus, by forming between the two ends of the incomplete vascular ring a compact mass of not easily compressed cells, the petiole can resist, though still with little effect, the intrusion and growth of the haustorium. In the region *s*, as shown in Fig. 2, sclerenchyma-fibres are formed in small groups; but in the section represented in Fig. 3 they are decidedly more numerous. The walls of these cells too are thicker and more strongly lignified. In other respects the characters and proportions of the component tissues of the petiole remain unaffected by the presence of the active haustorium.

We have seen that peripheral tissues difficult of penetration (such as those of *Aloë*, *Juncus*, *Equisetum*); that vascular bundles so scattered and so surrounded by resistant elements (as in *Juncus* and *Equisetum*) that the union of the haustoria with them is difficult and infrequent; that tissues containing poisonous matters (as in *Aloë* and *Euphorbia*), are great protections for a plant against the attacks of *Cuscuta*. Anatomical changes taking place after the haustoria have penetrated avail little, as the petiole of *Solanum* illustrates. But many plants resist attacks successfully merely because they are too large to be embraced by the parasite.

Unlike the Fungi, which find easier entrance and more suitable conditions for growth and development when a plant is ailing, *Cuscuta* flourishes the better in proportion as the hosts are stronger, healthier, more able to supply it abundantly with food without being forced soon to succumb to the constant drain. It is a distinct disadvantage to the Dodder when its host dies before it has itself come into flower and set seed. Its cycle of development is not so simple that it can rapidly be run through; it demands much food and strong mechanical support in order that its flowers may be advantageously displayed for the visits of insects, and that its fruits may successfully disseminate the seeds which they contain. Weak hosts cannot nourish well; hosts which easily succumb after being attacked do not afford the necessary mechanical support; and hosts which vegetate for only a short season, dying away either to the root or entirely, cannot assist in seed-dissemination.

II. The Penetration of the Haustoria into the Host.

1. *Penetration due to Mechanical Force.*

The various conditions affecting the origin and the development of the haustoria of three species of *Cuscuta* have been discussed in the foregoing pages. There remain to be considered in this paper the means by which the haustoria already formed in the parent plant make their way into the host and attach their xylem- and phloëm-elements to the corresponding elements of its vascular bundles. In my previous paper on certain phanerogamic parasites [1] my treatment of this part of the subject was regrettably incomplete, because, at the time of writing, only alcohol-material was at my disposal. From the living plants which it has since been my good fortune to be able to observe and to experiment upon, I am able to add to what I then wrote.

Manifestly in one or both of two ways only can the parasite

[1] Loc. cit.

ordinarily secure the penetration of its haustoria into a host; by mechanical pressure, or by chemical action, or by a combination of both. In my previous paper I expressed the opinion, based on the appearance of the excellent alcohol-material which I had examined, that the haustoria made their way by solution. I still believe this to be true, and I shall presently give some experimental evidence in favour of this view. I said nothing about pressure, for the evidences of pressure were very slight. The following experiments demonstrating that both solution and pressure accompany the intrusion of the haustoria into the host will show how necessary it was to supplement purely histological examination by physiological experiment. The plants used in the experiments now to be described were either *C. glomerata* or *C. europaea*, the plants of *C. Epilinum* by the time these experiments were begun having almost ceased to vegetate, developing only flowers and fruits.

If a branch of *C. glomerata* of suitable age be brought into contact with such a plant as *Zea Mais* at a part small enough in diameter to allow the branch to make close coils about it, haustoria will be produced. The branch should not be placed against the stem, which is protected by a peripheral ring of hard sclerenchyma, but rather against the base of a leaf still wrapped around the young stem, the whole diameter of stem and enclosing leaf being not more than one centimetre. After the haustoria first formed have penetrated the leaf, the leaf and the attached parasite should be cut away. Selecting, by the aid of a hand-lens, a haustorium which has developed to the point of entering the leaf but has not yet done so, careful transverse sections of the leaf should be made at this point, passing through and including the parasite which is attached to, though it has not yet penetrated into, the host. (The method of attachment is discussed in division II. 2 of this paper.) Taking for examination the section through the centre of the haustorium, the structure of the leaf will be seen to be as follows (see Pl. VIII, Fig. 4). The upper surface (the lower, *c–d*, in the figure), which so near the

base is of course the inner one and is more or less closely applied to the stem, consists of rather small thin-walled epidermal cells whose continuity is rarely broken by stomata. Immediately underlying this layer are the usual spongy parenchyma-cells which make up the larger part of the thickness of the leaf. At fairly regular intervals groups of these cells, about ten in each group, immediately underlying the epidermis, become more thick-walled and elongated, forming slender strands running lengthwise in the leaf, and thus adding something to its strength. The parenchyma-cells in the centre are large, thin-walled, spheroidal, and are adjoined by parenchyma-cells of similar character except that they are smaller in proportion as they are nearer the surfaces of the leaf. The under part of the leaf, the outer owing to its vertical position in embracing the stem, contains the large vascular bundles, whose course is longitudinal. These bundles project into the mesophyll, are separated from one another by considerable masses of parenchyma, and are surrounded individually by strongly lignified sheaths. The endodermis of each bundle is continuous with a plano-convex mass of sclerenchyma-cells which abuts by its plane side upon the epidermis. The epidermis of the lower side of the leaf consists of smaller cells with thicker walls, and is more frequently broken by stomata, than the epidermis of the other side. Except directly opposite the vascular bundles, where it adjoins the masses of sclerenchyma-cells, this lower epidermis, like the other, is in contact with the mesophyll-parenchyma.

Turning now to the parasite, we notice that the papillate cells of the epidermal cushion, which overlies the developing haustorium, have become so firmly applied to the epidermis of the leaf that their tips are flattened. The diameter of the whole cushion is about twice as long as the distance between two vascular bundles in the leaf. The young haustorium, developing in the cortical matrix of its parent, will be variously influenced by the resistance offered to the pressure which by its growth it exerts, and it will naturally, other

things being equal, grow in the direction of least resistance. Manifestly there will be less resistance to its forward growth midway between two vascular bundles of the Maize-leaf than directly opposite one of them; and against this intermediate region it pushes the epidermal and cortical cells which overlie it. It has been many times observed that even against strongly resisting rods of wood and glass the haustoria cause swellings of the stem by their growth. In the case now under discussion such a swelling directly over the tip of the haustorium is being pushed by the continued growth of the haustorium against the epidermal and parenchymatous tissues of the leaf which are situated between two vascular bundles (see Fig. 4). The epidermis of the leaf is pushed in by the low, blunt, conical swelling on the parasite, causing collapse of the larger, thinner-walled parenchyma-cells which are just behind it.

Passing to a somewhat older haustorium on the same coil, and sectioning it and the leaf to which it is attached, one sees that the continued and increasing pressure finally results in the rupture of the epidermal cells of the leaf. The epidermis is an elastic, tough, strongly-resisting layer. When it is broken through, the further progress of the haustorium by means of mechanical pressure is comparatively easy until it meets the thick-walled and strongly lignified cells of the endodermis of the bundles. In every experiment which I performed with *Zea* the haustoria failed to unite with the bundles; and hence the branches, which had been severed from the parent plants after the haustoria had penetrated the leaves, presently died.

Of course in the above case the pressure brought to bear upon the leaf was exercised by the haustorium only indirectly; it grew in size and length, and thereby pushed the adjacent cortical cells away from it; these in turn pushed forward the epidermal cells; these epidermal cells were rejuvenated by the contact and stimulated by the nutritious host to grow into papillae of very considerable size. In this cumulative manner a swelling arose on the surface of the parasite. By the con-

tinuation of these processes the swelling constantly increased in size, but more especially in height, and pressed the epidermal cells which had already been for some time in contact with the host more and more strongly against it. From their nature and arrangement the peripheral tissues of this host could not resist or transfer the pressure; they were forced to bend in and collapse; and so the pressure became evident. A moment's study of the much more delicate and yielding stem of *Impatiens Balsamina* will show how its peripheral tissues are affected by the pressure of the growing haustorium. Immediately underlying the single-layered epidermis which covers this essentially cylindrical stem, are five layers of collenchyma-cells which form a fairly strong and decidedly elastic ring about the deeper tissues. Within this ring are five or six layers of large rather thin-walled parenchyma-cells which in turn enclose the more or less continuous ring of vascular bundles. There is an abundant central pith. We see that the stem is composed of four concentric rings enclosing a core of pith. These rings are not rigid, for they are composed, except the wood, of elastic and not thick-walled cells with larger or smaller air-spaces between them. Even the wood is composed of comparatively thin-walled ducts and fibres, and is so frequently traversed by medullary rays that this ring also is anything but rigid. Hence pressure exerted upon one part of the stem can be transferred to and divided among all parts, a slight change of form of the whole stem allowing the cells in the region first pressed upon to escape for a considerable time much if not all injury from this cause. Furthermore, the structure of the stem is such that horizontal pressure is not only distributed among the cells on the same plane, but among those above and below also, and so its effects are rendered still less evident. If it were not that the host is tightly embraced within the close spiral formed by the parasite, we could conceive of its being able, by sufficient change of form, to escape being penetrated by the haustorium which, after all, is able to exert only a limited amount of pressure; but no stem strong enough to be

erect is able to do this, especially when it is enclosed by the parasite wound tightly and many times about it, for it is not yielding enough.

That the pressure exercised by the growing haustorium of *C. glomerata* is very considerable, though so completely masked by the yielding nature of the stems and petioles of the species of *Impatiens* upon which it is parasitic, may be readily demonstrated thus. Wind a sheet of tin-foil four or five centimetres long and only wide enough to meet, not to overlap, around a stem or erect branch of *Impatiens* of suitable size, fastening it by binding bast. Bring into contact with the stem thus enwrapped a healthy branch of the parasite. Close coils will be formed and haustoria will also be induced by the contact. Their formation and growth cause the usual swellings, and these press with constantly augmenting force against the tin-foil. Finally the foil is ruptured, tearing in irregular fashion, and thus allows (see Fig. 5) the epidermal cells covering the irregular protruding mass to come into contact with the host. The haustorium continues to grow and presently makes its way into the host. If the tin-foil be wound twice about the host, the haustoria fail to penetrate the foil, though making marked impressions in it; and they become abortive just as when they are formed in consequence of contact with other innutritious supports such as glass or wood. The tin-foil used in this experiment was two-tenths of a millimetre in thickness and of good quality. Through it the host could exercise no chemical influence on the parasite, nor could the parasite excrete a solvent of the metal. Hence we see that very considerable pressure is exerted by the growing haustorium and the epidermal cells immediately overlying it, and that pressure alone is sufficient to accomplish penetration into the host.

Such pressure is paralleled by that exercised by normal roots, whether they be lateral or main ones. Prunet[1] has again called attention to this in a paper on the penetra-

[1] Prunet, M. A., Sur la perforation des tubercules de Pomme de Terre par les rhizomes des Chiendents, Revue Générale de Botanique, III, 1891.

tion of potato-tubers by the rhizomes of 'Quitch Grass' (*Agropyrum repens*, Beauv.). From experiments of my own on seedlings of various plants producing both large and small main roots, it is perfectly evident that the power of penetrating living tissues by pressure alone is not a peculiarity of haustoria (which are but lateral roots modified in origin and structure to accomplish a very special purpose), nor of the roots of some Grasses. If a root be so firmly fixed against a plant that, if it grows at all, it can only grow into the opposing tissues, it will invariably penetrate them. Into how solid tissues these ordinary roots can make their way it is not within the scope of this paper to discuss. It is sufficient now to state that they readily penetrate through tissues as solid as the cortical tissues of any of the hosts of *Cuscuta* which I have yet seen. As recently determined by Pfeffer[1], the pressures which ordinary roots are able to exert are great. Accurate determinations of the pressures exerted by haustoria are, on account of the habits of the plants, most difficult to make; but from the celerity and apparent ease with which the haustoria of *C. glomerata* penetrate tin-foil it is hard to suppose that they are the weakest of all roots.

From the foregoing experiment it becomes evident that, though one object of the close coils formed about its host is to produce intimate contact of a considerable area of the parasite with its host, in order to induce the formation of numerous haustoria, there is still another and also a very important object. Owing to the closeness of the coils and to the large area consequently applied to the host, any force acting between the parasite and the host and tending to push them apart, would be resisted by the very great friction which must be overcome before the parasite could be made to uncoil. This resistance is not far from the breaking-strength of the stem. The force developed by the growing haustoria tends to push host and parasite apart, and it is resisted in this way. But the matter is not so simple. After a branch has made

[1] Pfeffer, W., Druck- und Arbeitsleistung durch wachsende Pflanzen. Abhandl. d. K. S. Gesellschaft d. Wissenschaften, XXXIII, 1893.

a close, nearly horizontal turn about the host, the curved part still continues for a time to grow in length. It is true that this growth is neither rapid nor of long duration, yet it is sufficient to loosen the spiral somewhat, to weaken the contact, to reduce the stimulus for haustorial formation, to lessen the force which the growing haustoria can bring to bear on the host, by increasing the distance through which they must grow. However, accompanying and following this comparatively slight growth in length is a very considerable and long-continued growth in thickness. The result of this latter is more than to counterbalance the growth in length. It maintains the closeness of the spiral, it constantly increases the intimacy of the contact, it strengthens thereby the stimulus for haustorial formation, and supplements the force which the growing haustoria can bring to bear on the host, by reducing the distance through which they must grow. This growth in thickness further supplements the force by (first) causing the stem of the parasite to press against and thus to compress the host by its whole concave side, and not merely by the swellings over the young haustoria; and (second) by increasing the rigidity of the coils so that, firmly braced by the coiled stem in which their strong bases are embedded, the haustoria can apply all their force forwards and against the host. Besides, the parasite is not merely closely pressed and firmly held against the host, but, as I shall demonstrate, is actually attached to it by many of the epidermal cells covering the swellings over the haustoria.

That the stem of the parasite, whether it bear haustoria or not, and whether it be coiled closely or only steeply and loosely around the host, exercises a very considerable pressure, might be suspected from its resemblance to tendrils and twining stems, but it may be made quite evident by the simple method used in demonstrating, though not thereby measuring, the pressure of tendrils, and the relative pressures developed by the two sorts of coils will at the same time be indicated. Let a branch of *Cuscuta* which is irritable, and therefore in condition to make close turns, be brought into

contact with a glass rod whose average diameter is known; and another branch, which is not in the irritable stage, be brought into such a relation with another rod of the same diameter that it will make steep turns about it. After both branches have twined about the supports for from thirty-six to forty-eight hours, remove the rods with as little disturbance of the coils as possible. It will at once be noticed that the diameter of both coils decreases, and that the number of turns increases proportionally. After waiting from twelve to twenty-four hours, the contraction will have reached its maximum and have become permanent. By measuring now the average diameters of the two imaginary cylinders enclosed by the gradual and the steep spirals respectively, by the close turns formed in consequence of irritation and by the loose turns formed without irritation (that is, as a climbing plant would form them), we find that the diameter of the former is from one-half to three-quarters the diameter of the rod first employed, whereas the diameter of the latter is never less than three-quarters the diameter of the rod. Evidently the steep spiral, formed only for supporting the plant as it ascends, exercises little if any more than enough pressure on the support to keep the plant from slipping down by its own weight; whilst the close spiral, formed in consequence of irritation for the purpose of forming an intimate and enduring contact and for enabling the haustoria the more readily to penetrate the host, exercises very considerable pressure. As shown before, the pressure exercised by the closely coiled stem is still more increased by the formation and growth of the haustoria.

But the force brought to bear on the host by the parasite, whether through the gradual or the steep spirals, is not merely one of compression; for by its circumnutation the parasite exercises a force which, unfortunately, has not yet been determined. We must therefore recognize two sorts of force which are applied to the host; a deflective, exercised by circumnutation, and a compressive, exercised by the coils and the haustoria growing in and from them. Doubtless the sum

of these two, when it is possible to estimate them in mechanical units, will be found surprisingly large when the delicacy of the plant developing them is taken into consideration.

2. *Penetration by the chemical activity of the pre-haustorium.*

Von Mohl [1] noticed that when a *Cuscuta* had wound about a polished silver rod, the positions of the haustoria against this rod were indicated, after the plant had been untwined from the support, by spots of a glairy fluid or of a shiny dry deposit. This he believed to be a mucilaginous matter which the parasite exuded in order the better to fasten itself to the host. L. Koch [2] considers it to be a solvent which is secreted for the purpose of softening if not dissolving the opposing tissues of the host, but he gives no experimental proof of this hypothesis. There are in the host three sorts of matter which may be affected by the parasite: the cell-walls, composed of cellulose and more or less infiltrated matter; the starch and other carbohydrates in solid form; and the nitrogenous substances. Though the haustorium may be able by mechanical pressure alone to penetrate the tissues and to bring its vascular elements into direct contact with the phloëm and xylem of the host, yet it goes without saying that it would be greatly to the advantage of the parasite were it able to supplement physical force by chemical action, and thus not only to make penetration easier, but also to allow the cells of the haustorium to dissolve, and thus to bring into available form, the solid nutritive matters around them. I have demonstrated in the foregoing pages that both contact and nourishment are necessary to the full development of haustoria. I shall now describe certain experiments showing some of the ways in which this nourishment is obtained.

Let a mixture of two parts plaster of Paris and one part of starch (I used the large-grained starches of potato and barley), very thoroughly stirred together, be wetted with

[1] Mohl, H. v., loc. cit. (cited by Koch on p. 55).
[2] Koch, L., loc. cit., 1880, pp. 56, 57.

a small quantity of water and cast into rods of about six centimetres length and less than one centimetre diameter. These may be sterilized just before using by soaking for fifteen minutes in ether and then rapidly evaporating under diminished atmospheric pressure. Into a moist chamber made of large glass-tubing, such as I have previously described (page 75), which has been thoroughly sterilized, insert the irritable tip of a branch of *C. glomerata*, and by sterilized forceps place the rod of plaster and starch in the chamber and in contact with the branch, quickly closing the chamber. In the course of six days the branch should have twined and developed several haustoria. Cutting the branch from its parent, remove it, still in contact with the rod, from the chamber. By a clean flat-pointed needle remove some of the starch and plaster from directly under a haustorium and examine it in water under a microscope. With another clean flat needle remove some of the starch and plaster from some part of the periphery of the rod far removed from any haustoria, and examine it also in water. It will be noticed that on the first slide, of material from under a haustorium, the proportion of starch to plaster is smaller than in the original mixture, and that most of the starch-grains still to be found are corroded in various degrees (see Pl. VIII, Fig. 6). The corrosion proceeds in the majority from the centre of the grain toward the periphery, forming broad and more or less radial canals; but a very considerable number of grains show the corrosion beginning at the periphery and running in, or beginning at several points between periphery and centre. In the last case, the corrosion proceeds from several centres instead of from one, but finally a common cavity is made by their union in the centre of the grain. Examination of the second slide, of material far from the influence of haustoria, will show that the proportion of starch to plaster is approximately the same as that of the original mixture[1], and that none of the starch-grains are corroded.

[1] It is scarcely to be expected that the ingredients should have been so perfectly mixed that their proportions would not vary somewhat in different parts of the rod.

If now the tip of a haustorial swelling be cut off and examined with a little of the starch and plaster still adhering, it will be seen that the haustorium has not penetrated the overlying epidermis, but that the epidermal cells have become papillate, and that most of the starch-grains still existing, either in contact with or very near the papillae, are deeply corroded. The corroded starch-grains can more accurately be observed, however, if after cutting off a bit of the stem in contact with the rod, some of the mixture still adhering to the tips of the haustorial swellings be removed to a slide, spread out thereon, and examined in a drop of water.

Evidently then the starch in contact with and in the vicinity of the papillate epidermal cells has been acted upon by them. That starch-grains not in contact with these cells are also acted upon shows that the ferment which is secreted is capable of diffusion for some distance through plaster of Paris, however limited may be its power of diffusion through colloids [1]. Examination of the plaster and starch with which unmodified epidermal cells are in contact, that is, between the haustorial swellings, shows no corrosion; the starch-grains are as unacted upon as those from parts of the rod far from the *Cuscuta*. The papillate cells are as well developed as those formed when the parasite is in contact with a host, showing that they have been nourished by what they have dissolved. The haustoria, though not so far advanced as they would be in the same length of time had a living plant been used instead of a rod, have yet developed farther than they would had an entirely insoluble and innutritious support like glass been used. The solvent action of the papillate cells results, therefore, in the acquisition of food used not only by themselves but by the haustoria also.

Since starch is contained within the cortical tissues of the host, it remains to be shown whether the papillate epidermal cells of the parasite reach this food-supply merely by rupturing

[1] Brown, H. T., and Morris, G. H., A Contribution to the Chemistry and Physiology of Foliage Leaves. Journ. Chem. Society, London, May, 1893, pp. 656–8.

the cells in which it is stored, or whether by a less violent process they are enabled to act upon it. One can demonstrate this by means of a slender stick of elder-pith impregnated with a nutrient solution, as was used in determining the conditions for haustorial formation (see p. 76). Let such a stick of pith be put in contact with the irritable part of a branch of *Cuscuta* enclosed in a moist chamber of large glass-tubing, quickly closing the chamber. After a few days, the *Cuscuta* having made many turns about the stick and bearing haustoria in several stages of development, one may sever the branch from the parent and remove it, still in contact with the pith, from the chamber. Sections through haustorial swellings which seem firmly fixed in some way to the pith, will demonstrate that the papillate epidermal cells are as large and healthy as normal, and that some of them have penetrated the cells of the pith to a greater or less depth, some even into the third row from the surface. These pith-cells seem little compressed, certainly not collapsed or ruptured. Clearing thin sections by means of equal parts of glycerine and water, and examining them under a high power of the microscope, we see that the epidermal cells have entered the cavities of the pith-cells through holes in the walls. These holes correspond with considerable accuracy to the shape and size of the cell or cells passing through them (Fig. 7). By staining with chlor-iodide of zinc and washing away the excess by distilled water before clearing in the mixture of glycerine and water, the result will be made still more evident; for the difference in composition of the cell-walls of the pith and of the *Cuscuta*, with the resulting differences in the shades of blue produced by the reagent, and the presence of protoplasm and nucleus (stained yellow or brown) in the cells of the parasite and the absence of these structures in the pith-cells, enables one to see very clearly that not by pressure alone could the epidermal cells have thus made their entrance. These epidermal cells, collectively forming the 'pre-haustorium,' must have secreted an enzyme which at least has softened and, since one can see no broken pieces of cell-wall turned back or pushed to one

side by the intruders, undoubtedly also dissolved the cellulose. By this solvent action exerted on the walls of the cortical cells of the host, the food-substances contained therein can be reached and be appropriated later by the papillate cells. The holes thus dissolved, constantly increasing in number and, as I shall presently show, in size by the solvent action of the cells at the tip of the haustorium proper, enable the young haustorium to grow rapidly forward through but feebly opposing tissues toward the conducting-tissues of its host. That the cellulose thus dissolved is probably consumed by the parasite is indicated by the normal development of the papillate epidermal cells, and the very considerable development of the haustoria formed by those branches twined about sticks of pith saturated with decoctions. For, though the decoction is nutritious at first, it so rapidly deteriorates in nutritive value that it can be useful for only a short time, and the pith, containing of itself no food of any kind except the cellulose of its walls, must be extremely innutritious.

The epidermis which covers a haustorial swelling consists of two sorts of modified cells: those lying directly over the haustorium become separate from one another, except at their bases, and grow out into long papillae: those surrounding them, and therefore not in, though parallel with, the line which the growing haustorium will follow in penetrating the host, elongate but do not separate from one another, and hence do not become papillate. The walls at their tips are very nearly as thin as those of the papillae. Both sorts of cells come into intimate contact with the walls of the epidermal cells of the host. The papillate cells exercise a solvent action so energetic that holes are made through the walls of those cells that oppose their further growth. The non-papillate cells, like the others in the kind, but not in the amount, of new growth which they accomplish, also excrete a solvent through their terminal walls, but the quantity of solvent excreted is not large. By this means only a partial solution of the walls of the epidermal cells of the host with which they are in contact takes place, but the walls of the contiguous cells of parasite and host

become fused together so perfectly that (Fig. 4) they are quite indistinguishable from one another. This union is very firm, as is shown by the resistance encountered in tearing the parasite away from its host even before the haustoria have penetrated. This union is sometimes broken after a time by the pushing apart of parasite and host owing to the forcible penetration of the haustorium through not readily soluble tissues, but in such cases the walls of the cells, once firmly united and then torn apart, are irregular and ragged.

I have already pointed out (p. 61) that the parasite can twine about leaves and send haustoria into them. The curve of the parasite about the lamina of a leaf can be only an ellipse. Manifestly in such a case as this there can be but little of the mechanical advantage which aids the haustoria in penetrating an approximately cylindrical stem. The stem of the parasite no longer furnishes an unyielding brace on which the haustoria are based; consequently it will be pushed away from the surface of the leaf by the growth of the haustorial swellings, just as the leaf too bends away under the same pressure. Evidently by pressure alone the haustoria would only with great difficulty penetrate the leaf. The rejuvenated epidermal cells of the parasite, however, perform the same work as before. The tips of the non-papillate 'cushion-cells' fuse with the walls (which they partially dissolve) of the opposite epidermal cells of the leaf, and the leaf is thus securely held. The papillate cells, collectively the 'pre-haustorium,' perforate the walls of the cells opposite them by a more complete solution than that accomplished by the 'cushion-cells,' and, growing through the holes thus made, enter the mesophyll. Held fast against the leaf by the 'cushion-cells,' and anchored by the pre-haustorial papillae, the stem of the parasite can now brace, and so assist, the haustoria in their forward growth. Although one 'cushion-cell' holds parasite and host but feebly together, yet there are so many of them that the attachment becomes very firm. The area of attachment is large; through the centre of this area the haustorium grows; its tip is conical, not blunt, and

hence meets with less resistance; its way has been partly excavated by the papillate cells. Hence the haustorium, growing along a partly made way in the line of least resistance, usually brings to bear on the attaching cells no more strain than they are able to bear.

We see in the results of the experiments just described additional reason for distinguishing in the 'cushion' of older authors which overlies the young and growing haustorium, two sorts of epidermal cells. The long papillate cells in the centre dissolve a passage for the permanent and much more effective haustorium proper; they use and transfer to other parts the nutritive substances which, after having dissolved them, they can readily absorb. Their functions are certainly those of a haustorium, and therefore I have ventured to call them collectively the 'pre-haustorium.' The other modified epidermal cells may justly retain the name of 'cushion-cells,' though, as I have just shown, their function is plainly that of hold-fasts.

We see, therefore, that Von Mohl's observation previously cited, that these cells pour out a secretion, was a correct one; but his opinion that the secreted substance is a mucilaginous matter was a mistaken one, though the object, that of cementing the two plants together, is attained by the partial solution accomplished by small quantities of the enzyme and the subsequent fusion of the two adjacent walls.

3. *Penetration by the chemical activity of the haustorium.*

Experimental demonstration that the haustorium itself exerts chemical, as well as mechanical, action on the cells which oppose its growth, is difficult, owing to the relatively innutritious supports composed of lifeless organic matter which are the only ones that may be used. For, though it seems improbable, yet it might be possible that living cells, irritated by the contact or the nearness of the intruding organ, would be stimulated into secreting a solvent by which their own walls would be broken down. Yet if such were really the

case we should expect to find not merely small parts of the wall, and these of shape and size proportioned to the attacking cells, but large portions gone, in fact a general disorganization of the tissue in the region attacked. No such changes in the attacked tissues of a host occur, and hence we cannot suppose that the host destroys its own tissues to any extent. That the haustorial cells are chemically active is strongly indicated in two ways. First, a study merely of alcohol-material inclines the observer to doubt that such accurate adjustment of the phloëm- and xylem-elements in the central cylinder of the growing haustorium with the phloëm- and xylem-elements of a vascular bundle of the host could be accomplished by mechanical means alone. How accurate this adjustment is, even to the correspondence of thick and thin areas in the walls of the haustorial tracheids with similar areas in the walls of the tracheae of the host to which they apply themselves, I have already demonstrated [1]. Second, thin sections of young haustoria of *C. glomerata* in the fleshy stems or petioles of *Impatiens Balsamina* show that the long papillate cells at the tips of the haustoria pass through the walls of the opposing parenchyma-cells of the host in the same way as do the papillate cells of the pre-haustorium.

In the experiment with elder-pith it is evident that the dead pith-cells cannot form new walls, but in a living plant one must determine accurately whether, when a papillate haustorial cell applies itself to a living parenchyma-cell of the host, the wall of the parenchyma-cell be simply pressed in by the force of the growing haustorial cell ; whether the natural extensibility of the cell-wall thus being gradually pushed into the cavity of the cell, be supplemented by growth leading to the development of the at first shallow depression into a cup, and finally a cylindrical pocket, enclosing the haustorial cell ; or whether there is actual perforation of the wall of the parenchyma-cell, and whether the thin wall of the papilla is in actual contact with the protoplasmic and other contents of the cell into which it has grown. A careful study of thin and

[1] Loc. cit. p. 300.

well-cleared sections of a young haustorium in the thick cortical parenchyma of a fairly large, fleshy stem, of *Impatiens* will show that the course of events is as follows[1]. A papillate cell at the tip of the haustorium becomes, by its growth in length, closely applied to the wall of a parenchyma-cell. By the pressure produced by more growth the tip is flattened against the opposing wall and becomes slightly enlarged. Presently the wall of the parenchyma-cell becomes gradually thinner and more incurved within the circular line described by the flattened tip of the pressing haustorial cell (see Pl. VIII, Fig. 8). When the haustorial papilla first comes into contact with the wall of the opposing parenchyma-cell, one can plainly distinguish the two walls from one another without the aid of staining agents; but by using a solution of the chlor-iodide of zinc the walls of the two cells become differentially stained. The wall of the cortical parenchyma-cell may be thicker than that of the haustorial cell, and may for this reason be more intensely stained; but besides this difference in quantity of colour, one sees also a difference in quality, for the cellulose of the younger papillate-cell becomes red purple, rather than blue purple, as the other does. When, however, the tip of the haustorial cell has been for a time pressed and thereby flattened against the wall of the parenchyma-cell, and the wall of the latter has begun both to bend and to grow thinner at the point of contact, the most careful staining and examination under a high magnifying power fail to reveal any line separating the two. The two walls, by the partial solution of that of the parenchyma-cell at least, and, I think, of that of the papilla also, have become fused into one (Fig. 8). Finally, by the combined action of solution and pressure, the tip of the papillate haustorial cell enters the cavity of the parenchyma-cell, pushing the protoplasm and nucleus to one side in some cases (Fig. 9), or more commonly penetrating, but otherwise not disturbing the protoplasm in any way. Furthermore, as Fig. 10 shows, the haustorial papilla becomes fused, at the region where it

[1] Compare pp. 307, 308 and figures 16ᵃ, 16ᵇ, and 16ᶜ in my former paper.

passes (at generally a right angle) through the wall of the parenchyma-cell, with the wall of that cell, so that not only does no opening by the side of the papilla exist, but it is impossible to determine exactly where the wall of the parenchyma-cell ends. The fusion is so perfect that the transition from older to newer cellulose, from parenchyma-wall to papilla-wall, is most gradual; they have modified each other in their perfect union. It seems to me by no means improbable, though micro-chemical and optical proofs are still wanting, that the rapid growth of the haustorial papillae is in part at least made possible by the walls which cover their tips not being quite solid; that indeed the enzyme which dissolves the walls of opposing cells also keeps the walls at the tips of the cells which secrete it in partial solution, and therefore in such condition that they but slightly resist the forward pressure from within. Careful staining by chlor-iodide of zinc, or by various agents producing more enduring stains (e.g. Congo Red), fails to show the slightest evidence that the wall of the cortical parenchyma-cell persists as a sheath around the papilla, and that the protoplasmic and other substances in its cavity are separated and protected from the haustorial cell by an involution of its wall. Its wall has become a constituent and indistinguishable part of the wall of the intruding cell, acting no longer as a protection against, but as a part of, the intruder.

We thus see that the haustorial cells penetrate those opposed to them. That this penetration is accomplished by means of chemical action as well as by pressure we see from the walls with which they come into contact becoming thinner at the points of contact as they bend in, and finally disappearing at these points, and from the fact that the holes through which the intruding cells pass are accurately proportioned to them in form and size. Through the repeated perforation of their walls by successive haustorial cells, the cortical cells which oppose the progress of the haustorium are removed, and along this tunnelled way the young haustorium reaches, and finally applies itself to, the conducting tissues of

the host. The starch-grains in the cells thus attacked and penetrated disappear, being dissolved and consumed by the protoplasm of the cells which contain them, or by the intruders; by which, it is of course impossible to tell.

We have seen that the penetration of the host by the parasite is begun by the pre-haustorium overlying the young haustorium proper, and that this is accomplished by the combined action of pressure and solution. The time and the field of action of the pre-haustorium are manifestly limited owing to its position, for the growing haustorium must finally crush and push through it. Some arrangement must be made to continue the work of the pre-haustorium, and this is effected by the cells at the tip of the haustorium, cells corresponding in position to those of the cap of a typical root. They become in appearance as in function like those composing the pre-haustorium, long, slender, thin-walled, with abundant protoplasmic contents and large nuclei, and great capacity for growth.

SUMMARY.

The results of the experiments described in the preceding pages may be briefly summarized as follows. The genus *Cuscuta* comprises parasitic climbing plants with two distinct modes of twining. At certain stages they resemble the majority of twining climbers in that they twine steeply, and only tightly enough to secure necessary mechanical support. They do not twine about horizontal rods whether they themselves be erect or horizontal. They twine in the direction in which they nutate; so far as I have been able to see, always in the reverse direction of the hands of a watch. At other stages, which regularly alternate with the foregoing, they make short, close, much more nearly horizontal turns about a vertical support, thereby embracing it closely and bringing their concave surfaces into intimate contact therewith. The former turns are made solely in consequence of the climbing habits of the plant, by the combined effects of circumnutation

and geotropism. The latter are induced by contact-irritation, which causes a modification of the manner and an acceleration in the speed of coiling.

Haustoria are ordinarily formed only upon the concave surfaces of the close coils. They also are the result of irritation, their formation being induced by contact of sufficient duration. Their development depends upon both contact and nourishment; without the one or the other only partial development takes place. The formation and perfect development of haustoria may take place on one as well as on another side of the stem, or on opposite sides at one and the same time, it being equally irritable on all sides, provided only the necessary contact and nourishment be supplied. They appear almost exclusively on the concave side of the close curves only, because there the contact-irritation is greatest; they develop best there because both contact and nourishment are on that side. Each haustorium is formed as a result of contact with an irritant object over the place of its origin. Innocuous liquids and wet gelatine are not irritant objects.

The periodic irritability of *Cuscuta* can be temporarily destroyed by revolving the plant horizontally around its long axis; that is, by neutralizing the effects of geotropism. In ordinary conditions geotropism is stronger than irritability, for the plant will not twine about horizontal supports; though, when a once vertical support about which a branch has closely twined is laid horizontally, the formation and development of haustoria already induced go on unhindered. In ordinary conditions these plants are not markedly heliotropic or hydrotropic; but when the effects of geotropism are neutralized by horizontal revolution on the clinostat, the plants become more sensitive to light and moisture. The comparative insensitiveness to light is not due to the absence of chlorophyll, nor is chlorophyll always absent. It is formed whenever for any reason the plant is insufficiently nourished, and the amount formed (that is, the intensity of the green colour) may be used as an index of the amount of organic food which it is receiving.

These parasites can attack successfully only those plants

whose size, peripheral tissues, internal structure, cell-contents and secretions, allow their being closely embraced by the parasites, their being readily penetrated by the haustoria, the speedy union of the conducting-tissues of the haustoria with their conducting tissues, while no poisonous effects are produced by cell-contents or secretions. The effects on the host are mainly physiological, it rarely happening that anatomical changes take place in consequence of the presence of haustoria.

Finally, the haustoria penetrate by means of mechanical pressure, of the chemical activity of the pre-haustoria, of the chemical activity of the cells at the tips of the haustoria proper; and these processes are aided, and in part shared in, by the cushion-cells.

I wish to express my warmest appreciation of and heartiest thanks for the numberless kindnesses which Professor Pfeffer and his assistants have constantly shown me in my work. Without their suggestions and criticisms but a small part of the investigation just described could have been accomplished.

LEIPZIG,
November, 1893.

EXPLANATION OF FIGURES IN PLATE VIII.

Illustrating Mr. Peirce's paper on *Cuscuta*. (Tissues of parasite red.)

Fig. 1. Part of petiole of *Solanum jasminoides* on which are scattered flower-clusters of a *Cuscuta*, the intermediate portions of the stem having atrophied and disappeared. Natural size.

Fig. 2. Outline of cross-section of petiole in Fig. 1, through line *a–b*, between two flower-clusters. *e*, epidermis; *c*, cortical parenchyma; *b*, phloëm; *x*, xylem; *s'*, sclerenchyma-mass; *s*, area in which are scattered sclerenchyma-masses; *p*, pith-parenchyma; *l*, bundle to leaflet; *H*, imbedded abortive haustorium. × 12.

Fig. 3. Outline of cross-section of petiole in Fig. 1, through line *c–d*, through a flower-cluster and one of its haustoria, *e*, *c*, &c., as above; except *p*, where parenchyma-cells have divided, become thicker-walled and lignified, under pressure. × 12.

Fig. 4. Cross-section of leaf of *Zea Mais* (*b*) showing pressure exerted by growing haustorium of *C. glomerata* (*a*); *c–d*, epidermis of upper (inner) side of leaf. × 120.

Fig. 5. Showing penetration by pressure of the growing haustorium of overlying cells through sheet of tin-foil: *a–b*, sheet of foil in cross-section: *C. glomerata*. × 120.

Fig. 6. Starch-grains of *Hordeum* corroded by cells of pre-haustorium of *C. glomerata*. × 490.

Fig. 7. Cross-section of elder-pith showing solution of its cell-walls by 'cushion-cells' of *C. europaea*. × 490.

Fig. 8. Cortical parenchyma of petiole of *Impatiens Balsamina*, in which wall of haustorial cell of *C. glomerata* has partly dissolved, bent, and is fused with wall of cortical parenchyma-cell. × 360.

Fig. 9. Showing penetration into cavity of parenchyma-cell by solution accomplished by haustorial cell, whose wall at *a* and *b* has fused with wall of parenchyma-cell. × 360.

NOTES.

A NEW CORDYCEPS.—A very remarkable species of *Cordyceps* has just been received at the Kew Herbarium from Owen's River, Victoria, where it was discovered by Miss M. Henley. It springs from a large caterpillar, and differs from all known species in the sharply differentiated fertile branches being erumpent from a simple, vertical stroma, eight to nine inches high; also in peculiarities of the ascophore. It will be known as *Cordyceps Henleyae*, and will be described in detail at a later date. G. MASSEE, Kew.

ABSORPTION OF WATER BY DEAD ROOTS.—Several experiments have already been made relative to the power of dead roots to supply nourishment to plants[1], leading to the conclusion that if the roots are killed without being ruptured, they may continue to take up moisture, and thus keep the plants alive for a considerable time. The matter may be roughly tested by killing the roots of the plants by immersing them for a time in boiling water, and carefully noting the results.

The following plants were experimented upon, with the result which any cultivator would have anticipated, namely, that dead roots are incapable of affording any continual sustenance to the plants to which they are attached. At the same time it is interesting to observe that in many cases the plants remained fresh for several days after their roots were killed. In every case care was taken to prevent the heat or steam from the boiling water from injuring the leaves or stems of the plants above the 'collar.' For the purpose of comparison the tops of two plants of *Cassia alata* were cut off level with the soil, and placed in water in the same house with those treated with boiling water. It will be seen that specimen 3, which had been severed under water remained fresh as long as the plant with boiled roots.

Cassia alata.—1. Plant in pot: roots immersed in boiling water on Feb. 16th, 1893; had not suffered on 20th; flagged on 23rd; leaves turned brown on 25th, and finally died.

[1] See Strasburger, Leitungsbahnen in den Pflanzen, Histologische Beiträge, III, p. 849, and the papers there cited.

2. Plant cut off level with soil on 17th, put in water instantly; flagged same day; leaves turned yellow by 23rd.

3. Plant cut off under water on 18th; older leaves turned yellow by 20th; flagged a little, and all leaves turned yellow by 25th.

'Purple Acacia' (Venezuela).—Plant shaken out of soil on 17th, and roots plunged in boiling water; by 20th some of leaflets had turned brown; on 21st the whole plant was flagging, and on 25th it was practically dead.

Bixa Orellana.—Plant in pot: roots immersed in boiling water on 16th; no signs of suffering on 20th; old leaves turned yellow by 25th; plant in a hopeless state by 30th.

Isotoma longiflora.—1. Plant in pot: boiled on 16th; flagging by 22nd; turned yellow by 25th.

2. All soil shaken off roots, roots plunged in boiling water; had flagged a little by 20th; leaves all turned black by 25th.

Tephrosia Vogelii.—Plant in pot: roots immersed in boiling water on 16th; leaves flagging by 22nd; turned brown by 25th.

Chrysanthemum indicum.—Plant in pot: roots immersed in boiling water on 16th; plant flagging by 24th; quite dead a fortnight later.

Poinsettia pulcherrima.—Plant in pot: roots immersed in boiling water on 16th; plant flagging by 20th; leaves all brown by 25th.

Livistona sinensis.—Plant in pot: roots immersed in boiling water on 16th; leaves shrivelling by 22nd; plant quite dead ten days later.

W. WATSON, Royal Gardens, Kew.

New Ferns of 1892–3.

BY

J. G. BAKER, F.R.S.,

Keeper of the Herbarium, Royal Gardens, Kew.

THE following are a number of new Ferns and Selaginellaceae which have been received at Kew from various sources during the last two years. The novelties sent home from Borneo by the Bishop of Sarawak and Singapore have been published in the Kew Bulletin, those obtained upon Kina-balu by Dr. Haveland were included in Dr. Stapf's paper read a short time ago before the Linnean Society, and the new species obtained in the Island of Grenada by Mr. R. V. Sherring have already been described in the Annals[1]. All the present species belong to genera already known, and do not furnish any striking peculiarity in structure which has not been already noted. The numbers which precede the names indicate their position in the sequence followed in our Synopsis Filicum, and for the two Selaginellas in my Handbook of the Fern-allies.

CYATHEACEAE.

29*. **Cyathea zambesiaca**, *Baker* n. sp. Frondibus amplis deltoideis tripinnatifidis modice firmis, utrinque viridibus, facie glabris, dorso pilosis, rachibus primariis inermibus sordide stramineis pilosis vix paleaceis, pinnis sessilibus lanceolatis

[1] See Annals, Vol. vi. 1892: also 'Summary of New Ferns,' in Vol. v. 1891, since published as a reprint.

rachibus pilosis, pinnulis sessilibus linearibus obtusis, inferioribus crenato-pinnatifidis, lobis rotundatis, venis pinnatis, venulis 4–5-jugis simplicibus erecto-patentibus, soris ad venas basalibus prope pinnularum costam uniseriatis, indusio late campanulate depresso membranaceo irregulariter rupto, receptaculo piloso.

Hab. Nyassa-land, *John Buchanan*, C. M. G.! Received in 1891, without number.

Pinnae inferiores subpedales. *Pinnulae* inferiores 15–18 lin. longae, 3–4 lin. latae.

Near *C. camerooniana*, Hook.

33*. **Cyathea phanerophlebia**, *Baker* n. sp. Frondibus amplis deltoideis bipinnatis subcoriaceis utrinque viridibus glabris, pinnis oblongo-lanceolatis, rachibus inermibus castaneis nudis, pinnulis lanceolatis sessilibus multijugis margine recurvatis deorsum integris superne obscure crenulatis, venis prominentibus crebris patulis prope basin furcatis, soris ad venas basalibus utrinque costam pinnularum crebre uniseriatis, indusio patellaeformi late explanato membranaceo glabro margine subintegro, receptaculo magno glabro.

Hab. North Madagascar, *Baron* 6109! Received Jan. 1892.

Pinnae pedales et ultra. *Pinnulae* 2–2½ poll. longae, 5–6 lin. latae.

A very distinct and handsome species, with the habit of *Alsophila Toenitis*.

50*. **Alsophila Ridleyi**, *Baker* n. sp. Frondibus amplis deltoideis tripinnatifidis rigidulis utrinque viridibus glabris, rachi nudo atrocastaneo inermi, pinnis oblongo-lanceolatis rachi facie piloso dorso castaneo glabro, pinnulis sessilibus linearibus subobtusis basi truncatis inferioribus ad medium pinnatifidis, lobis tertiariis oblongis integris, venis in lobis tertiariis pinnatis, venulis 4–6-jugis perspicuis arcuatis ascendentibus simplicibus vel infimis furcatis, soris medialibus.

Hab. Singapore, *Ridley* 4401!

Pinnae infimae pedales, pinnulis 1½–2 poll. longis, 4–6 lin. latis.

Allied to *A. comosa*, Wall. and *A. commutata*, Mett.

HYMENOPHYLLACEAE.

4*. **Hymenophyllum lindsaeoides**, *Baker n. sp.* Rhizomate filiformi nudo late repente, stipite brevissimo vel subnullo, frondibus lanceolatis glabris membranaceis pallide viridibus simplicibus crenato-lobatis ad basin angustatis, lobis uninervatis apice subtruncatis basi longe cuneatis, venis remotis erecto-patentibus in lobis centralibus, costa filiformi laminâ concolori, soris ad apicem loborum solitariis, indusio brevi marginali concolori, labiis semiorbicularibus erectis irregulariter denticulatis leviter inaequalibus, receptaculo incluso.

Hab. North-east Madagascar, *Humblot* 430! Received from Dr. Baillon in 1892.

Lamina 1½–2 poll. longa, medio 2–3 lin. lata.

A very distinct species, nearest to the well-known *H. asplenioides*, Sw., of the West Indies and Tropical America.

POLYPODIACEAE.

4*. **Lecanopteris incurvata**, *Baker* n. sp. Rhizomate ignoto, stipitibus brevibus erectis strictis sordide stramineis nudis, frondibus oblongo-lanceolatis simpliciter pinnatis caudatis membranaceis glabris facie viridibus dorso glaucescentibus, rachi primario basibus confluentibus pinnarum ad vel supra basin anguste alato, pinnis multijugis, sterilibus linearioblongis integris obtusis, fertilibus angustioribus lanceolatis profunde crenatis, venis in areolis hexagonis copiosis venulis liberis inclusis anastomosantibus, soris ad apicem loborum solitariis inflexis, indusio oblongo-naviculari unilaterali persistente, margine integro.

Hab. Sumatra, on the Barisan Mountains, between Kroe and Liwa, *Hancock* 88!

Lamina 10–16 poll. longa, 3–4 poll. lata. *Pinnae* steriles 6–7 lin., fertiles 3–4 lin. latae.

Nearly allied to *L. Curtisii*, Baker in Hook. Ic. t. 1607, from which it differs by its deeply crenate fertile pinnae and large inflexed indusia.

52*. **Davallia (Microlepia) firmula**, *Baker n. sp.* Stipitibus caespitosis elongatis gracilibus strictis supra basin stramineis nudis,

paleis basalibus parvis lanceolatis membranaceis ferrugineis, frondibus deltoideis glabris firmulis viridibus simpliciter pinnatis, rachi nudo gracili stramineo, pinnis sessilibus multijugis linearibus saepissime integris basi cuneatis, inferioribus haud reductis, venis laxis ascendentibus profunde furcatis ad marginem haud attingentibus, soris parvis uniseriatis ad venas apicalibus, indusio dimidiato cupulari glabro persistente.

Hab. Sumatra; Barisan Mountains near Liwa, alt. 2,700 feet, *Hancock* 72!

Stipites 8–9 poll. longi. *Lamina* 8–9 poll. longa et lata. *Pinnae* inferiores 5–6 poll. longae, medio 3 lin. latae.

Nearly allied to *D. pinnata*, Cav.

3*. **Adiantum gomphophyllum**, *Baker* n. sp. Stipitibus caespitosis erectis strictis nigro-castaneis subnudis, frondibus ligulatis simpliciter pinnatis utrinque glabris viridibus, rachi gracili nigro castaneo nudo, pinnis 5–6-jugis alternis breviter petiolatis deltoideis lateribus integris rectis apice lobato, lobis rotundatis crenulatis, venis flabellatis, soris ad pinnam saepissime 2 reniformibus ad apicem loborum impositis, indusio angusto glabro persistente.

Hab. Malay peninsula, on limestone rocks at Pungah, *Curtis* 2958!

Lamina 2–4 poll. longa, 8–9 lin. lata. *Pinnae* 3–4 lin. longae et latae, petiolis 1–1½ lin. longis.

Near *A. Gravesii*, Hance; Baker in Hook. Ic. t. 1632.

17*. **Asplenium (Euasplenium) spathulatum**, *Baker* n. sp. Stipitibus brevissimis paleis paucis parvis nigris lanceolatis praeditis, frondibus simplicibus membranaceis viridibus glabris supra medium oblongis cuspidatis superne crenulatis prope medium ad ligulam angustam integram cite spathulatim angustatis, venis parallelis leviter ascendentibus simplicibus vel furcatis ad marginem attingentibus, soris angustis elongatis ab marginibus remotis, indusio angusto glabro membranaceo.

Hab. Sumatra, four miles east of Bencolen, *Hancock* 31!

Lamina pedalis et ultra, supra medium 3–3½ poll., infra medium 6–9 lin. lata.

Nearly allied to the common American *A. serratum*, L.

Remarkable for the sudden narrowing of its fronds half-way up. Veins not very close.

51*. **Asplenium (Euasplenium) horizontale**, *Baker* n. sp. Stipitibus elongatis gracilibus viridibus minute paleaceis, paleis basalibus densis magnis lanceolatis acuminatis pallide brunneis, frondibus oblongis caudatis membranaceis glabris pallide viridibus, rachi viridi gracili parum paleaceo, pinnis 9 lanceolatis acuminatis obscure crenulatis horizontaliter patulis stricte sessilibus basi utrinque cordatis, auriculis supra rachin imbricatis, pinnis infimis maximis, venis subpatulis saepissime basi furcatis, soris medialibus linearibus, indusio lato membranaceo glabro persistente.

Hab. Sumatra, *Hancock* 59!

Stipites 8–10 poll. longi. *Lamina* pedalis. *Pinnae* 3–6 poll. longae, medio 10–12 lin. latae.

Allied to *A. sumatranum*, Hook. and *A. salignum*, Blume.

204*. **Asplenium (Diplazium) cordovense**, *Baker* n. sp. Stipitibus nudis elongatis brunneo-viridibus, frondibus oblongo-lanceolatis firmulis utrinque viridibus glabris dimidio inferiori simpliciter pinnatis dimidio superiori pinnatifidis, rachi nudo glabro viridulo, pinnis infimis subsessilibus oblongis acutis integris vel denticulatis basi inaequalibus antice productis, venis ascendentibus subtilibus furcatis, soris linearibus elongatis ad marginem haud productis inferioribus diplazioideis, indusio membranaceo glabro persistente.

Hab. Mexico; province of Cordova, *Hugo Finck*!

Lamina pedalis, basi 3–4 poll. lata. *Pinnae* infimae 2 poll. longae, 8–9 lin. latae. *Sori* centrales 4–5 lin. longi.

Allied to the Brazilian *A. Riedelianum*, Bong. and the Guatemalan *A. vera-pax*, Donnell-Smith.

206*. **Asplenium (Diplazium) confertum**, *Baker* n. sp. Stipitibus elongatis gracilibus viridibus supra basin nudis, paleis basalibus lanceolatis firmis castaneis, frondibus oblongo-lanceolatis simpliciter pinnatis firmulis utrinque glabris viridibus, rachi pubescente haud paleaceo, pinnis multijugis confertis sessilibus obtusis crenatis basi antice auriculatis, infimis paulo minoribus deflexis, venis obscuris erecto-patentibus furcatis, soris linearibus ab costa ad marginem

productis raro diplazioideis, indusio angusto glabro persistente.

Hab. Sumatra; Barisan range, thirty miles east of Bencolen, *Hancock* 15!

Stipites semipedales. *Lamina* 6–9 poll. longa, 2½–3 poll. lata. *Pinnae* 6–7 lin. latae.

Nearly allied to *A. porrectum*, Wall.

210*. **Asplenium (Diplazium) barisanicum**, *Baker* n. sp. Stipibus gracilibus elongatis nudis viridibus, paleis basalibus linearibus brunneis, frondibus oblongo-deltoideis simpliciter pinnatis utrinque viridibus glabris, rachi sursum pubescente, pinnis infra apicem frondis pinnatifidam paucijugis oblongo-lanceolatis profunde crenatis breviter petiolatis, basalibus haud vel vix minoribus, venis in lobis pinnarum pinnatis, venulis longis ascendentibus simplicibus, soris ad venulas basalibus ad apicem haud attingentibus raro diplazioideis, indusio membranaceo glabro persistente.

Hab. Sumatra; Barisan range, thirty miles east of Bencolen, *Hancock* 35!

Stipites semipedales. *Lamina* 7–8 poll. longa, 5–6 poll. lata. *Pinnae* 12–14 lin. latae.

Nearly allied to the Tropical American *A. grandifolium*, Sw.

220*. **Asplenium (Diplazium) shepherdioides**, *Baker* n. sp. Stipitibus elongatis gracilibus viridibus nudis, frondibus oblongo-lanceolatis bipinnatifidis utrinque viridibus glabris, rachi gracili nudo, pinnis lanceolatis acuminatis paucijugis ad medium pinnatifidis basi inferiori cuneatis superioribus sessilibus inferioribus breviter petiolatis infimis haud reductis, lobis secundariis oblongis integris, venis in lobis secundariis pinnatis venulis 4–6-jugis ascendentibus simplicibus, soris ad apicem venularum haud attingentibus raro diplazioideis, indusio angusto glabro persistente.

Hab. Sumatra; Barisan range, between Kroe and Liwa, *Hancock* 83!

Stipites 9–10 poll. longi. *Lamina* pedalis et ultra. *Pinnae* 3–5 poll. longae, 10–12 lin. latae, lobis 2–2½ lin. latis.

Near *A. Shepherdi*, Kunze and *A. speciosum*, Mett.

264*. **Asplenium (Anisogonium) Finckii**, *Baker* n. sp. Fron-

dibus dense caespitosis sessilibus lanceolatis integris acutis chartaceis utrinque viridibus glabris ad basin longe angustatis, costâ sensum gracili viridi deorsum brunnea crassiori, paleis basalibus densis lanceolatis atrobrunneis, venis ascendentibus in areolis hexagonis 3–4-seriatis anastomosantibus, venulis liberis inclusis nullis, soris brevibus ad dimidium centralem frondis solum productis, indusio viridulo glabro persistente.

Hab. Mexico; province of Cordova, *Hugo Finck*!

Lamina pedalis, medio 10–12 lin. lata, e medio ad apicem et basin sensim angustata. *Sori* inferiores 3–4 lin. longi, superiores breviores.

A very distinct novelty, with the habit of *A. angustum*, Sw., and the small forms of *A. serratum*, L. but with the veins anastomosing copiously in oblique hexagonal areolae.

30*. **Nephrodium (Lastrea) vulcanicum**, *Baker* n. sp. Stipitibus nudis gracilibus, frondibus oblongo-lanceolatis profunde bipinnatifidis utrinque praeter costam pinnarum glabris, rachi gracili viridulo persistente, pinnis sessilibus multijugis lanceolatis acuminatis ad alam angustam pinnatifidis, inferioribus haud reductis, lobis secundariis lineari-oblongis integris inferioribus valde reductis, venis in lobis secundariis pinnatis venulis 6–8-jugis obscuris immersis simplicibus, soris parvis medialibus, indusio parvo glabro persistente profunde reniformi.

Hab. Java; volcano of Pangerango, *Hancock* 69!

Lamina sesquipedalis, deorsum 5–6 poll. lata. *Pinnae* inferiores 7–8 lin. latae; lobis secundariis 1 lin. latis.

Near *N. chrysolobum*, Fée and *N. falciculatum*, Desv.

87*. **Polypodium (Dictyopteris) Hancockii**, *Baker* n. sp. Stipitibus strictis gracilibus nitidis nudis brunneis, frondibus deltoideis ternatim pinnatis membranaceis utrinque viridibus glabris, rachi nudo nitide castaneo, pinna terminali cordato-ovata medio profunde pinnatifida, pinnis lateralibus unijugis petiolatis ovatis valde inaequilateralibus postice productis conspicue auriculatis, venis primariis arcuatis ad marginem parallelis, venulis in areolis hexagonis copiosis anastomosantibus, venulis liberis inclusis copiosis productis, soris globosis superficialibus faciem totam pinnarum occupantibus.

Hab. Sumatra; Barisan range, between Kroe and Liwa, *Hancock* 89!

Lamina sesquipedalis, deorsum 10–12 poll. lata. *Pinna terminalis* 9–10 poll. longa, 6–7 poll. lata.

Habit of the large forms of *Aspidium trifoliatum*, with sori as in *Polypodium difforme*, Blume.

96*. **Polypodium (Eupolypodium) oblanceolatum**, *Baker* n. sp. Stipitibus brevibus filiformibus nudis, frondibus simplicibus oblanceolatis obtusis crenulatis membranaceis utrinque viridibus glabris paleis paucis brunneis subulatis praeditis ad basin sensim attenuatis, venis perspicuis laxe dispositis furcatis, soris superficialibus globosis inter costam et marginem medialibus uniseriatis ad furcam venarum productis.

Hab. New Guinea, Mount Suckling. Gathered by Sir W. Macgregor in 1892. Sent to Kew by Sir F. Mueller.

Lamina 1½–2 poll. longa, 2 lin. lata.

Near *P. ligulatum*, Baker and *P. subevenosum*, Baker.

101*. **Polypodium (Eupolypodium) oleandroides**, *Baker* n. sp. Stipitibus brevibus paleis subulatis brunneis recurvatis dense vestitis, frondibus lanceolatis subcoriaceis simplicibus integris obtusis ad basin attenuatis utrinque viridibus glabris margine paleis paucis brunneis subulatis ciliatis, venis perspicuis erecto-patentibus prope basin furcatis, soris globosis superficialibus confertis utrinque prope costam uniseriatis.

Hab. New Guinea; Mount Suckling, *Sir W. Macgregor*; received from Sir F. Mueller.

Stipites subpollicares. *Lamina* 5–6 poll. longa, medio 4–5 lin. lata.

Near *P. zeylanicum*, Mett.

107*. **Polypodium (Grammitis) sucklingianum**, *Baker* n. sp. Stipitibus brevissimis paleis subulatis brunneis patentibus vestitis, frondibus linearibus simplicibus crenatis obtusis ad basin angustatis utrinque viridibus glabris margine minute ciliatis, venis dissitis erecto-patentibus simplicibus ad apicem loborum productis, soris oblongis superficialibus obliquis utrinque prope cortam uniseriatis confertis.

Hab. New Guinea: Mount Suckling, *Sir W. Macgregor*; received from Sir F. Mueller.

Lamina 1½–2 poll. longa, medio 2 lin. lata.

Near *P. marginellum*, Sw. and *P. australe*, Mett.

111*. **Polypodium (Grammitis) ludens,** *Baker* n. sp. Rhizomate breviter repente, paleis basalibus densis late lanceolatis pallide brunneis, stipitibus productis paleis subulatis brunneis recurvatis praeditis, frondibus lanceolatis subcoriaceis utrinque nudis integris vel prope medium irregulariter lobatis ad basin angustatis, venis furcatis erecto-patentibus immersis obscuris, soris oblongis superficialibus obliquis utrinque prope costam uniseriatis.

Hab. Java, *Hancock* 53 a!

Stipites 2–3 poll. longi. *Lamina* 6–8 poll. longa, medio (lobis exclusis) 4–6 lin. lata.

Near *P. fasciatum*, Mett.

132*. **Polypodium (Eupolypodium) conjunctisorum,** *Baker* n.sp. Frondibus linearibus simpliciter pinnatis rigide coriaceis glabris ad basin sensim attenuatis, pinnis ovato-linearibus integris obtusis contiguis basi latis, venis immersis occultis, soris magnis globosis superficialibus ad basin pinnarum solitariis.

Hab. New Guinea; Mount Suckling, *Sir W. Macgregor*, received from Sir F. Mueller, March 1893.

Lamina 4–5 poll. longa, medio 3 lin. lata.

Near *P. moniliforme*, Lag.

139*. **Polypodium (Eupolypodium) malaccanum,** *Baker* n. sp. Stipitibus productis dense caespitosis gracillimis paleis brunneis subulatis patentibus fragilibus deciduis vestitis, frondibus lanceolatis simpliciter pinnatis modice firmis utrinque viridibus paleis subulatis brunneis ubique vestitis, pinnis multijugis linearibus integris obtusis basi dilatatis inferioribus minoribus, venulis simplicibus erecto-patentibus in pinnis centralibus 6–8-jugis, soris globosis superficialibus utrinque costam uniseriatis.

Hab. Gunong Mering, State of Malacca, *Ridley* 3345!

Stipites 1–2 poll. longi. *Lamina* 5–6 poll. longa, medio 5–6 lin. lata. *Pinnae* centrales 3–4 lin. longae, 1 lin. lata.

Near *P. parvulum*, Bory.

140*. **Polypodium (Eupolypodium) brachyphlebium,** *Baker* n. sp. Stipitibus brevibus gracilibus caespitosis paleis subulatis

brevibus patentibus proeditis, frondibus lanceolatis simpliciter pinnatis modice firmis utrinque viridibus pubescentibus ad basin attenuatis, pinnis contiguis multijugis linearibus obtusis integris basi late adnatis, venis brevibus erecto-patentibus simplicibus, soris globosis superficialibus utrinque costam pinnarum uniseriatis.

Hab. Sumatra; Barisan range, thirty miles east of Bencolen, *Hancock* 49!

Lamina semipedalis, medio 6–7 lin. lata, pinnis basi 1 lin. latis.

Near *P. parvulum*, Bory and *P. glandulosum*, Hook.

169*. **Polypodium (Eupolypodium) Macgregori**, *Baker* n. sp. Rhizomate brevi repente paleis basalibus parvis firmis densis erectis brunneis, stipitibus brevissimis strictis nudis, frondibus lanceolatis subcoriaceis glabris utrinque viridibus simpliciter, pinnatis ad basin sensim attenuatis, rachi nigro pubescente, pinnis linearibus integris obtusis basi late adnatis, venis immersis occultis, sōris globosis superficialibus utrinque costam uniseriatis.

Hab. Rossell Island, Louisiade Archipelago, *Sir W. Macgregor*, received from Sir F. Mueller.

Lamina 6–8 poll. longa, medio 3–4 lin. lata; pinnis basi 1 lin. latis.

Near *P. rigescens*, Bory and *P. blechnoides*, Hook.

171*. **Polypodium (Eupolypodium) stenobasis**, *Baker* n. sp. Rhizomate vix repente, paleis basalibus lanceolatis brunneis membranaceis, stipitibus subnudis, frondibus lanceolatis subcoriaceis elasticis simpliciter pinnatis utrinque viridibus glabris ad basin sensim attenuatis, costâ viridi pubescente, pinnis linearibus multijugis margine obscure crenatis basi late adnatis, venulis simplicibus obscuris occultis, soris utrinque costam pinnarum uniseriatis parvis globosis marginalibus profunde immersis.

Hab. Sumatra; Barisan range, thirty miles east of Bencolen, *Hancock* 51!

Lamina 6–9 poll. longa, medio 12–15 lin. lata, pinnis medio. 1 lin. latis.

Near *P. obliquatum*, Blume, but the sori marginal.

361°. **Polypodium (Phymatodes) sumatranum**, *Baker* n. sp. Stipitibus elongatis nudis stramineis, frondibus oblongo-lanceolatis profunde pinnatifidis modice firmis utrinque viridibus glabris basi vix angustatis, pinnis 8–9-jugis contiguis lanceolatis integris acutis basi in alam latam costularem confluentibus, venis in areolis hexagonis copiosis anastomosantibus, venulis liberis inclusis productis, soris magnis globosis superficialibus utrinque costam pinnarum 1–2 seriatis laxe dispositis.

Hab. Sumatra; Kephiang, Bencolen, *Hancock* 39!

Lamina 7–8 poll. longa, 3–4 poll. lata, pinnis medio 5–6 lin. latis.

Allied to *P. Phymatodes* and *P. Billardieri*.

384°. **Polypodium (Phymatodes) ludovicianum**, *Baker*, n. sp. Rhizomate longe repente, paleis lanceolatis membranaceis adpressis pallide brunneis vestito, stipitibus elongatis strictis erectis nudis, frondibus oblongo-deltoideis simpliciter pinnatis subcoriaceis utrinque viridibus obscure pubescentibus, rachi nudo glabro, pinnis 6–8-jugis lanceolatis integris haud contiguis basi late adnatis, venis obscuris immersis in areolis copiosis anastomosantibus, soris globosis leviter immersis utrinque costam bisereatis paginam totam demum occupantibus.

Hab. Louisiade archipelago, south-east island, *Sir W. Macgregor*; received from Sir F. Mueller.

Stipites 6–8 poll. longi. *Lamina* 6–8 poll. longa et lata. *Pinnae* inferiores 3–4 poll. longae, 3–4 lin. latae.

A well-marked species nearest *P. palmatum*, Blume.

SELAGINELLACEAE.

58°. **Selaginella (Stachygynandrum) Ridleyi**, *Baker*, n. sp. Caulibus continuis intricatis ad apicem decumbentibus, ramis superioribus remotis brevibus simplicibus prostratis, foliis seriei inferioris confertis patentibus oblongis obtusis deorsum ciliatis basi superiori productis cordatis, foliis seriei superioris duplo brevioribus ascendentibus ovatis conspicue mucronatis, spicis gracilibus tetragonis, bracteis parvis ovatis conformibus.

Hab. Gunong Mering, State of Malacca, *Ridley* 3346!

Rami foliati 2 lin. diam. *Spicae* 4–6 lin. longae, ½ lin. diam.

Near the Tropical American *S. jungermannioides*, Spring.

272*. **Selaginella (Heterostachys) oligostachya**, *Baker* n. sp. Caulibus continuis ad apicem radicantibus parce ramosis, foliis firmulis viridibus seriei inferioris ovatis subacutis patentibus haud contiguis haud ciliatis latere superiori productis basi cordatis, foliis seriei superioris ascendentibus parvis ovatis mucronatis, spicis brevibus, bracteis dimorphis, seriei inferioris parvis ovatis ascendentibus dense ciliatis, bracteis seriei superioris contiguis linearibus obtusis erecto-patentibus.

Hab. Gunong Mering, State of Malacca, *Ridley* 3347 !

Rami foliati 2 lin. lati. *Spicae* 6 lin. longae, 1 lin. latae.

Belongs to the small group of Bisulcatae, near *S. Kunstleri*, Baker and *S. gorvalensis*, Spring.

Contributions towards a Knowledge of the Anatomy of the Genus Selaginella, Spr.

BY

R. J. HARVEY GIBSON, M.A., F.R.S.E., F.L.S.,

Professor of Botany in University College, Liverpool.

With Plates IX, X, XI, and XII.

PART I.—THE STEM.

NOTWITHSTANDING the exceptionally important position which it occupies amongst Pteridophyta, and the interest attached to it phylogenetically, the genus *Selaginella* can scarcely be said to have received adequate treatment at the hands of comparative anatomists. The accounts of its structure given in the standard botanical text-books are based on researches made on a few of the more commonly cultivated species, e.g. *S. Martensii*, *S. caulescens*, *S. Kraussiana*, and *S. inaequalifolia*; and although references are not entirely wanting to the tissue-systems of other species, it must be admitted that a detailed account of the comparative anatomy of the genus is still a *desideratum*. Dangeard, it is true, has given us what he terms a 'Monographie anatomique des Sélaginelles' (22), where he treats of the structure of twenty-eight species, but his account of the anatomy is of the most

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incomplete character, scarcely any details being given either in text or plates. Moreover, he has fallen into many and serious errors. He has throughout made the crucial mistake (which others have made before him) of assuming that a section of the stem at any one level may stand for a section at any other, and that the structure of a lateral shoot-axis is necessarily the same as that of a primary erect axis or of the creeping rhizome. In the so-called polystelic species he has failed to determine accurately the course of the leaf-traces and the mode of anastomosis of steles at the ramifications. His monograph, in short, cannot be said to adequately fill the gap at present existing in our knowledge of this group of the Vascular Cryptogams, and does not compare favourably with the more special investigations of Treub, Vladescu, and others.

The diagnostic characters of the sub-genera and species have, as is well known, been almost exclusively based on external morphological features; and although I am far from desirous of minimizing the differences so emphasized, I feel convinced that anatomical structure, and especially the number and arrangement of the steles, must play *some* part in determining the relationships of the species. I trust that in the present and succeeding papers I may be able to offer some data on which generalizations of value may be based with regard to the phylogeny of the genus and its relations to other Vascular Cryptogams, both recent and fossil.

I have confined myself in the present contribution to giving an account of the anatomy and histology of the stem only; in a second paper, now in an advanced state of preparation, I hope to treat of the leaf, ligule, rhizophore, and root, reserving the cone and sporangia for a future contribution. The development of the embryo has already been investigated by Pfeffer (1) and Hofmeister (2), whilst Millardet (3) and Belajeff (29) have given an account of the development of the microspore and its contents. The special development of the vegetative organs has received considerable attention at the hands of Treub (14), Vladescu (21), Bruchmann (18), and others. Into

these developmental questions it is not my intention, at least in the present paper, to enter ; but rather to confine myself to the investigation of the anatomy of the mature plant. I shall have occasion in my next paper to refer to the structure of the growing apex in connexion with the mode of origin of the leaf and ligule.

In the naming of the species I have followed Baker's convenient manual (4), with reference also to other well-known systematic works, more especially those of Spring (6), Kuhn (9), Braun (7, 8), and M^{c}Nab (10). For convenience of anatomical comparison, however, I have grouped the species according to the number of steles in the erect shoot-axis, although I would wish it to be understood that this method of classification is for present convenience only, and does not *necessarily* express definite views as to the relationships of the species treated of.

I am greatly indebted to various botanists who have most kindly aided me in my work. I am more especially under obligation to Professor Dr. Graf zu Solms-Laubach, at whose suggestion and in whose laboratory in the University of Strassburg the work was first undertaken. I have to thank the Director of the Royal Gardens, Kew, for permission to carry on my work in the Jodrell Laboratory, and for access to the splendid collection of Selaginellas cultivated in the gardens. To Professors Farlow, Bower, and Bayley Balfour, and to Dr. King and Mr. Moore, my thanks are also due for fresh material from the Botanic Gardens of Harvard, Glasgow, Edinburgh, Calcutta, and Dublin respectively. I am further indebted to Dr. D. H. Scott, who has been so kind as to give me the benefit of his criticisms on several anatomical points. Lastly, I must record my acknowledgements to my Demonstrator, Mr. A. J. Ewart, B.Sc., for relieving me largely from the mechanical labour of imbedding and section-cutting. Messrs. Veitch, of Chelsea, have taken pains to supply me with much material which I required in duplicate.

The following list includes all the more important papers known to me as treating of the anatomy of the genus, more

especially those which deal with the structure of the stem. These papers, to avoid footnotes, are referred to in the text by the prefixed numeral.

1. Pfeffer. Die Entwicklung des Keimes der Gattung *Selaginella*. *Hanst. Bot. Abhandl.* 1871.
2. Hofmeister. Vergleichende Untersuchungen der Entwickelung höherer Kryptogamen. Leipzig, 1851.
3. Millardet. Le prothallium mâle des Cryptogames Vasculaires. Strasbourg, 1869.
4. Baker. Handbook of the Fern-Allies. London, 1887.
5. Spring. Monographie de la famille des Lycopodiacées. Pt. II. *Mém. l'Acad. roy. belg.* 1849.
6. Erikson. Bidrag till Kännedomen om Lycopodinebladens Anatomi. *Arbet. fran Lunds Bot. Inst.* 1892.
7. Braun. Beiträge zur Kenntniss der Gattung *Selaginella*. *Monatsber. Königl. Akad. Wiss. Berlin*, 1865.
8. ,, Appendices Plantarum quae in horto regio botanico Berolinensi coluntur, 1856–1867.
9. Kuhn. Filices Africanae. Leipzig, 1868.
10. McNab. On the Selaginellas of the Royal Botanic Gardens, Edinburgh. *Trans. Bot. Soc. Edin.* Vol. ix.
11. Russow. Vergleichende Untersuchungen über Leitbündel Kryptogamen. *Mém. l'Acad. Imper. S. Petersb.* Vol. xix. 1872.
12. Hegelmaier. Zur Kenntniss einiger Lycopodinen. *Bot. Zeit.* 1874.
13. Braun. Über die Blattstellung und Verzweigung der Lycopodiaceen. *Bot. Verh. Prov. Brand.* 1874.
14. Treub. Les organes de la végétation du *Selaginella Martensii*, Spr. Leide, 1877.
15. Sachs. Lehrbuch der Botanik, 1874 : 2nd English Ed. 1882.
16. De Bary. Vergleichende Anatomie der Gefässpflanzen, 1877. Eng. Ed.
17. Janczewski. Études comparées sur les tubes cribreux. *Mém. l'Acad. Nat. Sc. Cherbourg*, 1881.
18. Bruchmann. Die Vegetationsorgane von *S. spinulosa*, A. Br. *Zeitschr. f. Naturwissensch.* Halle, 1884.
19. Haberlandt. Die Chlorophyllkörper der Selaginellen. *Flora*, 1888.
20. Leclerc du Sablon. Sur l'endoderme de la tige des Sélaginelles. *Jour. d. Bot.* 1889.

21. Vladescu. Communications préliminaires sur la structure de la tige des Sélaginelles. *Jour. d. Bot.*, 1889.
22. Dangeard. Essai sur l'anatomie des Cryptogames vasculaires. *Le Botaniste*, Vol. I. 1889.
23. Wojinowić. Beiträge zur Morphologie, Anatomie und Biologie der *Selaginella lepidophylla*, Spr. *Inaug. Diss.* Breslau, 1890.
24. Strasburger. Über den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. *Histolog. Beitr.* III. 1891.
25. Van Tieghem. Traité de Botanique. 2nd Ed. Paris, 1892.
26. ,, Péricycle et Péridesme. *Jour. d. Bot.*, 1890.
27. Bower. On the structure of *Lepidostrobus Brownii*, Sch. *Ann. Bot.*, Vol. VII, 1893.
28. Harvey Gibson. On the siliceous deposit in certain species of *Selaginella*. *Ann. Bot.*, Vol. VII, 1893.
29. Belajeff. Antheridien u. Spermatozoiden der heterosporen Lycopodiaceen. *Bot. Zeit.*, 1885.

Historical Summary of Researches on the Anatomy of the Stem.

A considerable number of papers have been published on the anatomy of *Selaginella*; but these, as has been already stated, deal with only a few species. Moreover, the more important of these monographs treat either of the development of the vegetative organs, or of the special anatomy and development of the sporangia and their contents. Quite recently the structure of the leaf has received some attention from Erikson (6).

Spring's classic monograph (5) is so well known to all students of the Vascular Cryptogams that I need not do more than briefly refer to that part of his work which deals with the subject of the present paper. After an exhaustive discussion of the external morphology of 209 species of *Selaginella*, Spring gives an organographical summary of the family of the Lycopodiaceae, in which *Selaginella* is compared with the other genera which he includes under the group. Amongst other points he insists on the importance of considering the sectional outline of the stem in the determination

of species, and draws attention to the distinction to be made between the creeping and erect portions of the shoot-axis, a distinction which has been strangely lost sight of by later anatomists. Indeed, judging from the published papers, the creeping stems of some of the most important species, such as *S. spinosa*, *S. Braunii*, *S. inaequalifolia*, and *S. Lyallii*, seem never to have been sectionized at all. Spring remarks that the stem may contain one, two, four, or more vascular bundles, and adds that the so-called articulations on the erect stems of those species known as 'Articulatae' are due to hypertrophy of the cortex—the vascular system taking no part in their formation. The branching, according to Spring, is always dichotomous, a statement, however, which has not been confirmed by later researches. The 'corps ligneux,' always a multiple of two in number, is enveloped by a 'couche génératrice' of cellular tissue, which Spring considers as destined to become on the one hand 'liber,' on the other 'fibres ligneux.' The 'couche génératrice' is surrounded by 'liber' composed of long thin-walled cells, and that in turn by an 'enveloppe herbacée,' the cells of which contain chlorophyll, starch, &c., and an epidermis. Spring's 'couche génératrice' would apparently correspond to what is now considered true phloëm, the 'liber' being the so-called pericycle. No mention is made of the lacunae or of trabecular tissue; but in all probability Spring's observations were made on herbarium material, under which circumstances the lacunae might possibly be set down to the effect of desiccation.

Russow (11) states that the vascular bundles of *Selaginella* are built upon the fern-type, each consisting of one or more marginal protoxylems and a centripetally developed metaxylem of scalariform tracheides, surrounded by one or more layers of parenchyma (Geleitzellen). These layers separate the wood from the phloëm, which, he says, consists in greater part of a two to three-layered phloëm-sheath, enclosing an incomplete layer of proto-phloëm. No mention is made of sieve-tubes as such; indeed, he expressly says, 'deutlich ausgeprägte Siebgefässe finden sich nur bei Equisetum und

Ophioglosseen.' The ground-tissue is sharply marked off from the vascular system, but a true endodermis is not present. The vascular bundle is, however, slung in a lacuna by longer or shorter cuticularized cells, or the space may be filled with parenchyma. This trabecular tissue Russow looks upon as being the analogue of an endodermis. The cortex is composed externally of sclerotic fibres forming a supporting ring just beneath the epidermis.

Hegelmaier (12) discusses the nature and mode of origin of the cuticular band on the endodermal cells, pointing out that quite close to the growing apex the cuticular ring has not yet appeared, the whole wall giving a blue reaction with chloro-zinc-iodine. Shortly afterwards, however, the cuticularization appears, and the wall in that region gives a brown or violet reaction with that reagent. My own observations on the mode of origin of this peculiar ring differ somewhat from those of Hegelmaier. I have given these in detail in the course of my description of *S. Martensii*. I have found the method advocated by Hegelmaier, viz. boiling in caustic potash, most useful in isolating the vascular system and tracing the courses of the leaf-traces and protoxylem-cords.

Braun (13) has with justice drawn attention to the existence of a third type of leaf in the heterophyllous species, viz. the axillary leaf (Achselblatt). The importance of this leaf will be seen later in the discussion of the insertion of leaf-traces on the steles, more especially in the tristelic species.

Treub's developmental researches (14) may be best summarized in his own words :—'Il suit de la description que je viens de donner que les branches du *Selaginella Martensii* sont les monopodies qui, se ramifiant périodiquement, forment deux rangées de membres latéraux, et *non* des sympodes provenant de dichotomies successives.' He describes in some detail the development of the various layers of the stem, agreeing with Russow in considering the lacunar tissue as belonging to the fundamental tissue-system, an opinion which has been confirmed by the more recent researches of Vladescu and Strasburger. He differs from Russow in believing that

the phloëm contains true sieve-tubes. The bundles are cauline at first, and derived from elongated procambial cells; leaf-traces are inserted later on the margins of the bundle. Although I am not at present prepared to speak on the development of the tissues in *Selaginella*, still I may say that no tracheides appear above the point of insertion of the youngest leaf-trace. The cell-layer which gives rise to the lacunar tissue, Treub describes as dividing tangentially into 1°, a layer next the vascular system, the cells of which develope local annular cuticularizations on their walls, and 2°, an outer layer whose elements divide radially.

The accounts given by Sachs and De Bary may next be referred to. Sachs (15) draws attention to the absence of intercellular spaces in the cortex (a statement which, as will be shown hereafter, is by no means of general application) and to the cauline nature of the vascular bundles on which the leaf-traces are afterwards inserted. His account of the anatomy of the stem is fundamentally that given by Russow.

Further details are given by De Bary (16). He follows Russow in describing the vascular bundle as of the fern-type, and like that author considers a true endodermis to be absent. Probably the majority of the species have, De Bary says, one axile ribbon-shaped bundle, some having on the middle of the upper surface a median ridge. The leaf-traces are inserted on the margins of the axile strand. *S. Kraussiana*, *S. Galeottei*, and others are noted as having two axillary bundles. (This, however, is true only of the region between the points of origin of branches, and in some species even there the steles are connected by pericyclar tissue). Reference is also made to *S. inaequalifolia* with three vascular bundles (there are frequently as many as five, and these gradually fuse into one in the creeping portion of the stem) and to *S. Lyallii* with 'ten or twelve bundles distributed in three equidistant rows in an almost quadrate surface' (this is true only of the upper parts of the erect shoots), and to *S. spinosa*, where the single axile bundle has leaf-traces inserted on it all round. (Here again the creeping portion of the axis has an entirely distinct

structure.) De Bary adds that 'the course of the bundles and the insertion of the bundles of the leaves have not been investigated in those shoots which have other than one axile bundle or two lateral ones.'

In Janczewski's monograph (17) on the structure of sieve-tubes some details are given of the structure of these elements in *S. Martensii*. De Bary had remarked (16, p. 182) that 'the position in which the sieve-tubes occur in the above instances (Ferns) is occupied by elements of similar form and general character of contents and walls, but without distinct sieve-plates or sieve-pores.' Janczewski gives an accurate account of the structure of the vascular system of *S. Martensii*, and shows that genuine sieve-tubes with sieve-plates do exist in the situation indicated by De Bary. My observations on many species entirely agree with those of Janczewski.

Bruchmann publishes a short account (18) of his researches on *S. spinulosa*, A.Br., in which he states that the apical region of the stem and branches is occupied by a group of cells, not a single cell as Treub had demonstrated for *S. Martensii*. The first branching of the embryo of this species is, according to Bruchmann, a true dichotomy, but all subsequent branchings are monopodial. It is curious that all anatomists who have investigated *S. spinosa*, P.B. (*S. spinulosa*, A.Br.) seem to have missed the very peculiar alteration which the stele undergoes in the transition from the creeping to the erect axis.

Haberlandt publishes an exhaustive treatise (19) on the structure and development of the chlorophyll-bodies of the genus, to which I need not at present refer beyond saying that the completeness of his work enables me to omit detailed description of the chloroplastids in those layers of the stem which contain them.

A short note by Leclerc du Sablon (20) recurs to the question of the homology of the cuticularized cells which arise from the limiting layers of the vascular cords. He expresses it as his opinion that these cells form a genuine endodermis.

In the same year and in the same journal Vladescu pub-

lishes an important preliminary note (21) on the development of the various layers of the stem, in which he claims to show a common origin for the so-called pericycle, endodermis, and a certain number of the inner layers of the cortex. Each endodermal cell, he says, articulates with two cells of the pericycle on the one hand, and with two cells of the cortex on the other. As Vladescu does not state on which species his observations were made, it is necessary to say here that by no means all species have their endodermal cells so articulated. Indeed, the case varies in the same plant, and it would be strange, *a priori*, if any such arrangement were constant, since, according to Vladescu's own researches, the inner cortex, trabeculae, and pericycle are only modified layers of the general cortex. Vladescu believes that the endodermis of *Selaginella* has a conductive function.

Next in order of publication comes the paper by Dangeard (22) already mentioned. In this memoir the author, after briefly reviewing a few of the previously published researches, proceeds to discuss the anatomy and histology of twenty-eight species of *Selaginella*. To this part of his work I shall refer later on under the respective species. I may say, however, generally, that he gives little or no details of the minute structure of the tissue-elements, and the distribution of the constituents of the phloëm is not described save in the most casual way. Moreover, these points are not shown in his figures, the accuracy of several of which I must call in question. In the second part of his paper a comparative summary is given of the results obtained, the chief of which are as follows:—The epidermis is cuticularized, and the lumina of the epidermal cells are often obliterated. The conjunctive tissue is differentiated into a ring of stereome, a layer of polyhedral cells, and the layers of the inner cortex next the lacuna. The endodermal cells are provided with cuticularized rings, and are attached by one end to two cells of the limiting layer of the vascular cylinder, and by the other to two cells of the cortex. He often figures the endodermal cell as articulating with one cell on either side—his

figure being in that respect more accurate than his text. In the structure of the stele he distinguishes, although he does not figure, protophloëm and metaphloëm, protoxylem and metaxylem. He describes the metaphloëm as 'generally' consisting of two layers, one of which consists of fascicular parenchyma next the wood, the other of sieve-tubes with larger lumina. The modification 'generally' requires explanation; if it means that there is 'generally' one layer (one or more cells deep) of parenchyma next the wood, and a layer (in the same sense) of sieve-tubes without, then the statement might have been made absolute, for that is the arrangement in all the species; if, on the other hand, 'layer' means a *single* layer of cells, then the term 'generally' is inaccurate, for very few species have their metaphloëm so much reduced. No help is to be obtained from his figures, which frequently represent steles with far fewer layers in the phloëm than they really possess. In none of his figures does Dangeard distinguish between parenchyma and sieve-tubes, nor does he state whether his sections are taken from young or from old stems, or from the creeping or from the erect portions of the axis. The limiting layers of the vascular cylinder are usually known as the pericycle, but Dangeard, although accepting the conclusions arrived at by Vladescu with regard to the origin of these layers, goes further, and remarks, 'Il nous paraît difficile de séparer cette assise du liber avec lequel elle se confond parfois, et, en attendant le travail définitif de M. Vladescu, nous la rattacherons au liber sous le nom de périphragme.' I am quite unable to follow him in this; indeed, the so-called pericycle is, in my experience, the easiest layer to determine in the whole anatomy. The fact that its cells contain chlorophyll is alone sufficient to enable one to clearly differentiate pericycle and phloëm. Moreover, Vladescu's observations, which are accepted and confirmed by Strasburger (24), demonstrate a quite distinct origin for the phloëm. Dangeard, finally, proceeds to tabulate the species according to the number and arrangement of the steles; to this part of his work I shall refer in my general summary.

In a promotion-thesis Wojinowić (23) gives an account of the structure of *S. lepidophylla*, treating of it, however, rather from the biological than from the anatomical standpoint. A discussion of his work may more appropriately come in connexion with my own description of the stem in that species.

Strasburger (24) gives a summary of our knowledge of the structure of *S. Martensii*, in which he accepts Vladescu's account of the development of the stem. I shall have occasion presently to refer to his views on the homology of the various layers which surround the vascular cords. He for the first time draws attention to the occurrence of silica in the cortex of *S. Martensii*.

The second edition of Van Tieghem's Text-book (25) gives perhaps the latest authoritative general account of the structure of the genus (for Frank's more recently published *Lehrbuch* contains merely a repetition of Sachs' account). According to Van Tieghem the epidermis has no stomata, and the cortex is without intercellular spaces. As I have already pointed out, this latter statement is inaccurate, at least so far as the inner cortex of many species is concerned. There is, he continues, one central cylinder with two wood bundles enclosed by bast, and a single or double pericycle. In certain species (e.g. *S. spinulosa*, A.Br.) four protoxylem-bands occur. Van Tieghem has obviously not cut serial sections of the complete stem of that species, else he would certainly have arrived at a different conclusion. In the polystelic species he says there are three parallel binate bands (e.g. *S. inaequalifolia*), the median one alone giving attachment to the leaf-traces. Both of these statements are inaccurate; the steles are not binate (in the sense of having two protoxylem-strands—although that is obviously the interpretation of the well-known woodcut in Sachs' Text-book, reproduced by Van Tieghem), and the leaf-traces of the axillary leaves are inserted on the ventral stele. Moreover, the young creeping stem is monostelic, and the erect shoots may have as many as five steles. He then goes on to say that in some species the stele is separated into

its constituent bundles, each with an endodermis and pericycle; a binary stele so divided occurs in *S. Kraussiana*, *S. Galeottei*, &c.; two binary steles splitting into their constituent bundles occur in *S. Lyallii*. This account (p. 1435) does not seem to harmonize with that given at p. 766, where *S. Kraussiana* is spoken of as bistelic, *S. inaequalifolia* as tristelic, and *S. Lyallii* as polystelic. Van Tieghem also speaks of the bast as being interrupted opposite the protoxylem-strands, and a similar statement is also made by Strasburger (24). As a matter of fact the phloëm-parenchyma is almost never interrupted (exceptionally so in the creeping axis of *S. spinosa*), and the sieve-tube layer only in some cases. Even in *S. Martensii*, as has been pointed out by Janczewski, isolated sieve-tubes occur in the neighbourhood of the protoxylem.

Bower, in a recent paper on the structure of the axis of *Lepidostrobus* (26), makes some observations on the cortical tissue of *Selaginella*, and comes to the conclusion 'that the trabecular development in *Selaginella* is a specialized and more definite example of that lacunar development which appears in such various forms and positions in cortical tissues of various other Lycopodinous plants,' a conclusion with which I entirely agree.

In the same journal I endeavoured (27) to trace the development and distribution of the silica, which was known to occur in the cortex of *S. Martensii*, and which I have been able to demonstrate in several other species.

From the above summary it will be seen that our knowledge of the anatomy of the stem of this genus has not advanced to any appreciable extent since the date of De Bary's *Vergleichende Anatomie*, save perhaps in regard to the developmental origin of the layers, and what we owe to the careful researches of Janczewski on the nature of the sieve-tubes. That much yet remains to be done must be apparent to any one who has had the opportunity of examining types so divergent in anatomical structure as *S. spinosa*, *S. Braunii*, and *S. Lyallii*. I do not profess to have exhausted the subject even of the stem-structure, but I hope the

following pages will be found to contain a record of new facts, both anatomical and histological, which may serve to some extent as a starting-point for further investigation.

Before entering on the detailed discussion of the species, it appears to me advisable to give a brief outline of the signification I attach to the terms employed in this paper. This seems the more necessary, as botanists who accept the terminology advanced by Van Tieghem as applied to the vascular system and its enveloping layers, and the morphological views which such a terminology implies, are in the genus *Selaginella* brought face to face with a condition of things which in description requires very careful consideration.

I have designated throughout the limiting layer of the stem *epidermis*, although in many respects, notably the entire absence of stomata, the lignification of the cell-walls and its developmental origin, it differs from the layer of cells usually bearing that name. The sclerotic tissue which in the great majority of the species lies immediately within the limiting layer, I have termed either *stereome* or *hypodermis*. It must be understood however that, inwardly, this layer, in most cases at all events, merges gradually into the non-sclerotic cells of the general cortex. There is no difficulty so far: it is when one begins to discuss the inner layers of the cortex, the trabecular tissue and the limiting layers of the vascular system, and again the morphological value of the vascular strands themselves, that one finds considerable confusion of nomenclature, not to say diversity of opinion. Vladescu (21) has advanced certain views as to the mode of origin of the tissues in question. This author, as already stated, finds that the segment-cell derived from the apical cell divides so as to form ultimately three cells, the innermost being the parent of the xylem-elements, the enveloping parenchyma and the phloëm; the outermost cell gives rise to the epidermis and the outer cortex, whilst the median cell gives origin to the tissue which encloses the phloëm, the

cuticularized endodermal cells and certain layers of the inner cortex. Strasburger (24) describes the vascular cylinder as enclosed by one or two layers of cells which stand in place of the pericycle but actually belong to the rind. He goes on to say (*l.c.* p. 457): 'Alle diese Schichten (pericycle and endodermis) und auch noch die nach aussen folgende, welche die Trabeculae ausserhalb der Endodermis aufbaut, sind auf die Theilung der ursprünglich innersten Rindenschicht, des Phleoterma, zurückzuführen.' He adds, 'ich hatte keinen Grund, die inzwischen erschienenen Angaben von Vladescu anzuzweifeln.' As I am not prepared to express a personal opinion on the developmental origin of these layers, although, like Strasburger, I have no ground for doubting Vladescu's results, I have retained the terms 'pericycle,' &c. with the following explanation. By *endodermal cells* I mean the cuticularized cells which arise from the chlorophyllaceous layer surrounding the phloëm, and use the term *trabecula* in a general sense for the uni- or multi-cellular strands which anchor the vascular cords to the cortex. The trabecula is sometimes merely an endodermal cell; at other times one, two, or more parenchymatous cells are connected with the endodermal cell to form the trabecula. Similarly I retain the name *pericycle* for the green, externally cuticularized layer (or layers) which give origin to the endodermal cells on the one hand and enclose the phloëm on the other. For the semi- or wholly occluded bright-walled elements occurring immediately within the pericycle I have retained Russow's term *protophloëm*. The parenchymatous layer next the xylem is the next point on which explanation is necessary. For this tissue I have adopted the name *phloëm-parenchyma*, without expressing any opinion as to its developmental origin.

The final point on which I wish to make an explanation is with regard to the use of the terms 'vascular bundle' and 'stele.' I use the term *vascular bundle* to indicate a leaf-trace only, and retain the term *stele* for the vascular strand, enclosed within a pericycle and endodermis (using these

terms in the sense defined above). For instance, in the so-called bistelic species, such as *S. Kraussiana*, two such steles each with one protoxylem-group occur between the origin of branches, but at the origin of a branch these become united, i. e. gamostelic. On the other hand, one might consider the condition of the vascular tissue at the origin of a branch as monostelic, becoming between the origin of branches schizostelic. I have already pointed out that Van Tieghem himself seems to me not to be consistent in his use of the term stele in reference to *Selaginella*, and although I do not feel at all satisfied with the terminology, still in the absence of a genuine pericycle in the anatomical sense, I cannot see how the determination of what constitutes a true stele in this genus can be arrived at without reopening the subject of the stele as an anatomical unit—a matter foreign to the purpose of this paper. Personally I do not think perfectly safe conclusions can be arrived at without careful examination of the primary embryonic axis—not the growing-point of a principal or secondary shoot of the adult. It is possible, for instance, that developmental evidence may be forthcoming to show that in the primary embryonic axis the stele is surrounded by a genuine pericycle—the erect secondary shoots becoming schizostelic or meristelic, the separate bundles or groups of bundles being surrounded by a layer of specialized cortical tissue which may be considered as analogous to pericycle and endodermis. In any case, I am disposed to think that the morphological value of these sheathing layers is liable to be overrated. Professor Bower informs me that an endodermis is well marked in the rhizome of *Helminthostachys*, that it is not continuous either as a general or special sheath in *Botrychium Lunaria*, although present at the periphery of the bundles of the axis. *Ophioglossum vulgatum* and *O. reticulatum*, he also informs me, have doubtfully an endodermis, and *O. Bergianum* certainly wants it. Such cases as these, together with others such as that of *Equisetum*, make one chary of attaching very great weight to the presence or absence of sheaths, and in the

present paper therefore I employ the terms 'pericycle,' 'endodermis,' and 'stele,' *purely in a descriptive sense.*

Of the 334 species of *Selaginella* recorded by Baker (4) I have examined fifty-three (not counting numerous varieties) in a fresh condition. In the great majority of cases I was able to obtain the entire plant—not the erect shoots alone. This is of fundamental importance, since, as I shall show later on, the procumbent or horizontal axis in very many cases differs markedly in structure from the erect axis. I have divided the paper into two parts; the first deals with the anatomy and histology of the individual species, the second attempts to give a comparative summary of the general anatomy and histology. In the first section I have grouped the species round certain type-forms which are treated of rather more fully. For instance, a large number of species are closely related to *S. Martensii*, all characterized by the possession of a dorsiventral axis and a single ribbon-shaped stele. Since these are the forms most fully investigated I have dealt with them first. *S. oregana* serves as the type of that section which is characterized by having homophyllous leaves and yet a ribbon-shaped dorsiventral stele. Certain anomalous monostelic forms are then discussed, such as *S. spinosa*, *S. Braunii*, &c. The bistelic species are associated with *S. Galeottei* as the type, and the tristelic species with *S. inaequalifolia*. *S. Lyallii*, perhaps the most aberrant of all, I have dealt with in a special section.

Section I. Anatomy of Species.

A. *Martensii Type.*

1. *Selaginella Martensii*, Spr. Baker's Handbook, No. 179.

The stem of this well-known and often investigated species is partly trailing, partly ascending, but in all parts dorsiventral. One ribbon-shaped stele runs throughout the axis, lying in a lacuna sharply marked off by siliceous deposit

on the cortical wall. There are two marginal protoxylems formed by fusion of the leaf-traces of the dorsal and ventral leaves of either side, connected in the young condition by procambium, later by metaxylem. A dorsal strand of protoxylem occurs on the stele for some little distance beneath the origin of a branch, and is formed by the fusion of the adjacent marginal protoxylems of the branch and chief axis. This cord soon fuses with the marginal protoxylem of the axis beneath on the same side as that on which the branch arises (Pl. IX, Fig. 1).

The stem is covered externally by a cuticle and epidermis, followed by a cortex composed outwardly of elongated sclerotic fibres, medianly of long thin-walled cells, polyhedral in section. The inner cortex consists of smaller cells with minute intercellular spaces in which a deposit of silica occurs. As I have described this siliceous incrustation in this and other species in a previous number of this journal (28) I need not do more than simply indicate its presence.

The lacuna is large and well defined, and is crossed by trabeculae of two types. The first and simpler type consists of an endodermal cell with a cuticular band, articulating on the cortical side with two swollen cells which arise from adjacent cells of the inner cortex. The other type of trabecula is derived from this by the swollen cortical cells undergoing subdivision, so that the endodermal cell articulates with a cluster of cortical cells. These cells contain chlorophyll and have a siliceous deposit in the spaces which occur between them. I have endeavoured to trace the origin of the cuticular band on the endodermal cell, and the conclusions I have arrived at are somewhat different from the views expressed by Hegelmaier, Treub, and others. According to the accepted account the cuticular band is local in origin. *A priori* it is somewhat difficult to see why it should be so, and more especially when the position of the band in the young state is compared with that in the old. It should also be borne in mind that if the views of Vladescu and Strasburger be correct with regard to the homologies of these cells, viz.

that they are only peculiarly modified cells of the inner general cortex, and not the specialized innermost layer of the cortex usually termed endodermis, then one would not so anxiously seek for the characteristic local thickenings on the radial walls. In any case my observations do not lead me to believe that the cuticularization is at all local in origin, but rather that it is an isolated product of the cuticle which uniformly covers the outer layer of the pericycle. As figured by Treub, the first stage in the development of a trabecula is the isolation (by a surrounding air space) of a cell stretching from the layer which will become the pericycle to the cortex. The pericycle has even at this stage a very thin cuticle. Subsequently the cuticle creeps up and invests the mother-cell of the trabecula, or rather the inner of the two cells into which the mother-cell has by this time divided. This inner cell is the future endodermal cell. The outer cell then divides by a radial division plane into two cells whose outer ends next the cortex remain narrow, but whose inner terminations swell considerably and form two balloon-shaped cells articulating with the endodermal cell (Pl. IX, Figs. 3–5). These then separate from each other at the cortical side and then through their entire length. Meanwhile differential growth causes elongation of the endodermal cell, so that the cuticularization becomes isolated as a ring. As has been pointed out by Strasburger (24), older endodermal cells may shew partial or complete cuticularization as a secondary phenomenon. This is what one would expect to take place when differential growth has ceased. Where the endodermal cell runs straight across the lacuna the annular band is median or nearly so, owing to equal growth in extent of the endodermal cell-wall between the annulus and the cortex on the one hand and the pericycle and annulus on the other. In the creeping axes of *S. Lyallii*, *S. inaequalifolia* and other forms where practically no lacuna is developed, and between the primary and accessory steles of many primarily tristelic species, the endodermal cells or their analogues are completely cuticularized, or at all events exhibits no cuticular annulus. I look upon this peculiar cuticularisation merely as a special

case of that general cuticularization which takes place on the walls of cells exposed to air and the annular nature of the cuticularization as a secondary phenomenon resulting from the elongation of the endodermal cell following on the excessive development of the lacuna in this region. It ought to be remembered in this connexion that the endodermal cells contain no chlorophyll, although the pericycle on the one side and the inner cortical cells on the other both contain chlorophyll. Vladescu suggests that these endodermal cells are conductive in function; they are undoubtedly supporting as well.

The pericycle is one layer deep, sometimes two, and contains chlorophyll (Pl. IX, Fig. 2). [It ought to be stated that in this and all subsequent cases the sections described were taken from stems of as nearly as possible equivalent age.] The protophloëm elements are few in number. The elements described by Janczewski as occurring just within the pericycle and as having the sieve-tubes radially arranged round them, are, it seems to me, simply protophloëm-elements in process of occlusion. The sieve-tubes are two layers deep dorsally and ventrally, although opposite the marginal protoxylem-areas one imperfect and broken layer occurs. One or two layers of elongated parenchyma separate the sieve-tubes from the xylem, which latter is composed entirely of tracheides.

Accounts fundamentally similar to that given above have been published by Strasburger, Janczewski, Treub, and others.

2. *Selaginella grandis*, Moore. Baker's Handbook, No. 243.

This large and beautiful species has a short decumbent stem, rooted at close intervals, from which thick erect shoot-axes arise. These are unbranched for a foot or so and then are copiously branched and dorsiventral. The creeping portion and erect shoots are fundamentally similar in structure.

There is one large ribbon-like stele bearing marginal and dorsal protoxylems, as in *S. Martensii*, and arising in a quite similar manner to those in that species. There is an epidermis and cuticle, a thick hypodermis, and a cortex of thick-walled pitted cells. Those next the lacuna are narrow and tubular, deeply pitted and enclosed in a siliceous deposit. These cells

peel off the cortex proper and articulate in pairs with the endodermal cells (Pl. IX, Fig. 7).

The pericycle is two to three layers deep opposite the margins of the stele, and three to four layers deep dorsally and ventrally. The sieve-tubes are two layers deep dorsally and ventrally, but one layer only occurs near the margins, and they are entirely absent opposite the protoxylems. The xylem is large in amount and forms a broad thin band. Procambial tissue is present even in well-developed xylems. The xylem is separated from the sieve-tubes by two to four layers of small, much elongated parenchyma.

3. *Selaginella Vogelii*, Spr. Baker's Handbook, No. 250.

Both creeping and erect shoots of this species are monostelic, and the course of the leaf-traces and protoxylems on the stele are as in *S. Martensii*. Older stems have no trichomata on the epidermis, but young branches have a plentiful development of hairs on the ventral surface. All these hairs point towards the apex of the branch. They are unicellular and strongly cuticularized; their bases are swollen and they taper to a fine point.

In section the stem is nearly circular and is covered by a cuticle and epidermis of the usual type. A considerable amount of stereome occurs in the erect shoots, but the creeping stems have little or none. The cortex is thin-walled, and the cells, especially in the procumbent parts, contain large quantities of starch. The cells of the cortex are smaller in diameter towards the lacuna. The trabeculae consist of endodermal cells articulating with one or more parenchymatous cells continuous with and creeping over the inner cortex (Pl. IX, Fig. 8). The pericycle is three to five layers deep. There are many crushed protophloëm-elements dorsally and ventrally, but these are absent at the margins of the stele. The sieve-tubes are absent opposite the marginal protoxylems; elsewhere they are two layers deep. They are separated from the xylem by three to four layers of parenchyma.

4. *Selaginella haematodes*, Spr. Baker's Handbook, No. 261.

This species is in many respects like *S. Vogelii*. The

protoxylems are arranged on the plan of those in *S. Martensii*, and the creeping and erect stems are both monostelic. There is externally a cuticle, a scarcely differentiated epidermis and a thick hypodermis, the thick cell-walls of these layers being of a bright red colour. The cortex is abundant and the walls of the cortical cells are also thick. The innermost layers are composed of tubular cells with thick sclerotic walls and are enclosed in a siliceous deposit (Pl. IX, Fig. 9). Then follow two or more layers of loosely arranged thin-walled chlorophyll-bearing parenchyma with silica, articulating with the endodermal cells. These latter are short and usually much constricted in the region of the cuticular band.

There is a single ribbon-like stele with two marginal protoxylems. The pericycle is three layers deep dorsally and ventrally, but two-layered opposite the margin. Two layers of sieve-tubes occur dorsally and ventrally, and one imperfect (or occasionally perfect) layer opposite the marginal protoxylems. There are a few protophloëm-elements. The phloëm-parenchyma is well developed and from three to four layers deep.

5. *Selaginella erythropus*, Spr. Baker's Handbook, No. 260.

The stele is simple throughout and the arrangement of protoxylems typical. There is a cuticle, epidermis, and about twenty layers of stereome-cells. The inner layers of the cortex are thin-walled, and a small siliceous deposit occurs on their walls. The endodermal cells articulate with one or more swollen green cells lying loosely against the innermost layers of the cortex. The pericycle is from two to six layers deep in the fully developed erect shoot. There are two layers of sieve-tubes, absent however opposite the marginal protoxylems, and separated from the xylem by two to four layers of phloëm-parenchyma. The cortex of the creeping stem is composed entirely of sclerenchyma, whose walls are deeply pitted, both lumina and pits being filled with a red colouring substance (Pl. IX, Fig. 10).

6. *Selaginella Douglasii*, Spr. Baker's Handbook, No. 56.

This species resembles in some respects *S. helvetica*, Lk., but the dorsal cord soon fuses with the marginal protoxylem.

The epidermis is distinct and encloses a poorly developed hypodermis. The inner cortex has small intercellular spaces. The trabeculae consist of endodermal cells articulating with two united strings of parenchyma-cells gradually merging into the inner cortex. The pericycle is two layers deep at the margins of the stele, and three layered dorsally and ventrally. One or two layers of sieve-tubes occur dorsally and ventrally, but these elements are absent opposite the margins of the stele. Two or three layers of parenchyma separate the sieve-tubes from the xylem.

7. *Selaginella caulescens*, Spr. Baker's Handbook, No. 232.

The arrangement of protoxylems in this large and well-known species approaches closely to that seen in *S. Martensii*, only the dorsal cord does not fuse so quickly with the adjacent marginal protoxylem. I have examined, in addition to the type species, the varieties *japonica*, *minor*, and *argentea*, and find no substantial anatomical differences between them and the type. There is a cuticle, epidermis, and thick hypodermis, a thick cortex of cells polyhedral in section, ceasing abruptly at the margins of the lacuna. A siliceous deposit occurs in some of the varieties, though not, so far as I have seen, in *S. caulescens* itself. Longer or shorter endodermal cells articulate with swollen chlorophyll-bearing cells on the cortical side. The pericycle is two to five layers deep, being thinner opposite the margins of the stele. There are a few crushed protophloëm-elements within the pericycle. The sieve-tubes are arranged in one or two layers dorsally and ventrally, and are separated from the xylem by two to four layers of parenchyma. The metaxylem is abundant save in the horizontal axes, where it is small in amount or altogether wanting.

8. *Selaginella Griffithii*, Spr. Baker's Handbook, No. 237.

This species is anatomically very like *S. Martensii*. There is a well-marked epidermis, four to five layers of stereome, merging gradually into a thick-walled cortex, sharply marked off internally by a siliceous deposit. The stele is simple, with two marginal protoxylems and a dorsal cord

formed, as in *S. Martensii*, out of the fused adjacent marginal protoxylems of branch and axis. The pericycle is two to three layers deep. The trabeculae are similar to those in *S. Martensii*. The sieve-tubes are two layers deep save opposite the marginal protoxylems, where there is only one (occasionally interrupted) layer. The sieve-tubes are separated from the xylem by two to three layers of phloëm-parenchyma (Pl. IX, Fig. 11).

9. *Selaginella Karsteniana*, A. Br. Baker's Handbook, No. 332.

The anatomical and histological structure of the stem of this species is almost identical with that of *S. haematodes*, and does not therefore require special description. A deposit of silica occurs on the inner cortex.

10. *Selaginella plumosa*, Bak. Baker's Handbook, No. 65.

The arrangement of the protoxylems is on the normal plan. The pericycle is two to three layers deep, enclosing one layer of sieve-tubes absent opposite the marginal protoxylems. Two to four layers of parenchyma enclose the xylem. The endodermal cells arise from single cells of the pericycle, and end in clusters of cells lying against the inner cortex.

Dangeard figures the course of the protoxylem strands in *S. monospora*. *S. monospora* is, according to Baker, a variety of *S. plumosa*. Dangeard makes the inner marginal protoxylem of the branch unite with the outer marginal protoxylem, the adjacent marginal protoxylem of the chief axis alone forming the dorsal protoxylem-cord of the stele. I have not had an opportunity of examining *S. plumosa*, var. *monospora*, and so can only say that in *S. plumosa* itself the arrangement of the protoxylems is on the *Martensii* plan.

11. *Selaginella suberosa*, Spr. Baker's Handbook, No. 318.

The protoxylems are arranged on the *S. Martensii* plan. There is a distinct epidermis followed by two or more layers of stereome. The cortex consists of large cells, and there is a deposit of silica on the innermost layer. The endodermal cells are short and articulate with several swollen cells which

almost fill the lacuna. The stele is slender and consists of a pericycle of one or two layers of large cells, one or two layers of sieve-tubes, absent opposite the marginal protoxylems and not infrequently interrupted dorsally and ventrally as well. There are numerous protophloëm-elements. The sieve-tubes are separated from the xylem by a single layer of parenchyma.

12. *Selaginella stenophylla*, A. Br. Baker's Handbook, No. 334.

This species has the anatomical characters of *S. suberosa*. The sieve-tube layer on the dorsal side is frequently interrupted by parenchyma. The ventral layer is, however, continuous. There is a small amount of silica on the inner cortex. The pericycle consists of one layer of large cells, and the parenchyma next the wood is two to four layers deep. In specimens in my possession the rhizophores are in the upper parts frequently replaced by normal leafy shoots.

13. *Selaginella viticulosa*, Klotz. Baker's Handbook. No. 258.

This and several of the succeeding species, though differing much from *S. Martensii* in habit and general morphology, yet do not vary greatly from it in anatomical structure.

The stem of *S. viticulosa* is rooted in the lower part, ascending and dorsiventral above, but is fundamentally the same in structure throughout. The stele is simple and ribbon-like, and the leaf-traces are inserted on the marginal protoxylems as in *S. Martensii*. There is one (sometimes two) dorsally situated protoxylems derived from the fusion of the adjacent marginal protoxylems of primary and secondary axes, the dorsal cord of one side not fusing with the adjacent marginal protoxylem until after the formation of a second dorsal cord from the fusion of a second branch on the opposite side. I cannot confirm Dangeard's figure of the course of the protoxylems. (22. Pl. XII, Fig. 1).

The stem is on the whole quadrangular in section. There is an epidermis and cuticle,—the epidermal cells being thin-

walled and elongated save near the bases of the leaves, where they are short, thick-walled, and deeply pitted. There is a hypodermis of from eight to ten or more layers of sclerenchyma, containing a small amount of chlorophyll. The cortical cells are large and densely packed with very large chloroplastids and starch. The trabecular tissue consists of short endodermal cells articulating with from two to five long twisted cells, which are in turn continuous with the loosely arranged inner cortical cells. These latter cells are also long and tubular, and contain abundant starch and chlorophyll (Pl. IX, Figs. 12, 13). The pericycle is two layers deep at the margins of the stele, and three layers deep dorsally and ventrally. The cells contain chlorophyll and the external layer has as usual a well-developed cuticle. A few crushed protophloëm-elements occur beneath the pericycle. There is only one layer of small-lumined sieve-tubes, completely interrupted opposite the marginal protoxylems. The sieve-tubes are separated from a normal xylem by from three to four layers of parenchyma, the cells of which are very long and narrow.

14. *Selaginella serpens*, Spr. Baker's Handbook, No. 50.

The entire stem of this species trails along the ground, rooting at intervals from the points of origin of branches. The stele is simple throughout, and the arrangement of leaf-traces and of the dorsal protoxylems resembles that in *S. Martensii* (Pl. IX, Fig. 14). The stem is covered by a cuticle and epidermis, the cells of which have narrow lumina and contain chlorophyll. There is a hypodermis consisting of from one to three layers of sclerenchyma. The innermost layer of the cortex is continuous with the distal cells of the trabeculae, which consist of endodermal cells articulating with one or two chlorophyll-bearing cells. The endodermal cells are very much elongated just at the origin of branches (Pl. IX, Fig. 15). There is a pericycle of one to two layers of large cells covered externally by a cuticle. The sieve-tubes are one layer deep and quite absent opposite the marginal protoxylems. Occasionally the sieve-tube layer is

broken dorsally and ventrally as well. One to two layers of parenchyma separate the sieve-tubes from the xylem.

15. *Selaginella involvens*, Spr. Baker's Handbook, No. 204.

The dorsal cord in this species soon fuses with the marginal protoxylem. There is an ill-defined epidermis and a plentiful development of stereome. The cortex is composed of thick-walled tubular cells, with intercellular spaces in which an abundant deposit of silica occurs (Pl. IX, Fig. 17). The inner layers are loosely arranged and continuous with cells which articulate with the short endodermal cells. The stele is convex dorsally and flat ventrally, and composed of a two to three layered pericycle of small cells, one to two layers of sieve-tubes absent opposite the marginal protoxylems and separated from the xylem by, on an average, three layers of parenchyma.

16. *Selaginella cuspidata*, Lk. Baker's Handbook, No. 213.

The arrangement of the leaf-traces and protoxylems is of the usual type. The stem has externally a cuticle, epidermis, and three to five layers of sclerenchymatous hypodermis. The cells of the inner cortex are small, loosely arranged, and have a siliceous deposit in the minute intercellular spaces. The trabeculae are not numerous and consist of endodermal cells articulating with the loose creeping inner cortical cells. These cells are full of starch and chlorophyll. The pericycle is as a rule one layer thick, though here and there on the dorsal and ventral surfaces it is double. One layer of sieve-tubes occurs dorsally and ventrally, but they are absent opposite the marginal protoxylems. The phloëm-parenchyma is three to four layers deep. Amongst the scalariform tracheids there occur shorter elements with irregular reticulate thickenings.

17. *Selaginella mollicops*, Spr. Baker's Handbook, No. 325.

The arrangement of protoxylems is normal. There is a cuticle, large celled epidermis, but almost no hypodermis. The inner cortical cells are loosely arranged, those next the lacuna being swollen and articulating with the endodermal cells. The lacuna is large and the stele lies loosely in it.

The pericycle is two layers deep, though here and there one large cell replaces two smaller ones (Pl. IX, Fig. 16). There are a few crushed protophloëm-elements. The sieve-tubes are very variable in diameter and one layer deep. The largest tubes occur dorsally and ventrally, the smallest ones nearer the margins of the stele. One or two layers of parenchyma occur next the wood.

18. *Selaginella apus*, Spr. Baker's Handbook, No. 144.

This small trailing species has the normal arrangement of leaf-traces and protoxylems. The dorsal cord unites almost at once with the marginal protoxylem of the side on which the branch arises. There is a large-celled epidermis but almost no stereome. The cortex is four to six cells deep, the cells being large and thin-walled. The innermost layers are loose and articulate with the endodermal cells. The pericycle is one layer thick. One layer of sieve-tubes occurs dorsally and ventrally, but is absent opposite the marginal protoxylems. One to two layers of parenchyma surround the xylem. Dangeard (22) remarks that the metaxylem is 'nul ou presque nul.' The metaxylem is certainly small in amount but is never wanting save in the growing apices, where of course it is replaced in all species by procambium.

19. *Selaginella lepidophylla*, Spr. Baker's Handbook, No. 211.

The arrangement of the protoxylems is normal. There is an epidermis scarcely distinct from the very thick hypodermis. The walls of the epidermal and hypodermal cells are very thick and deeply pitted (Pl. XII, Figs. 106–107). The cells contain a considerable amount of oil, but very little chlorophyll. The xylem has the normal marginal and dorsal protoxylems. The xylem is surrounded by two or more layers of parenchyma, and one or (rarely) two layers of dorsiventrally flattened sieve-tubes, absent opposite the marginal protoxylems. The sieve-tubes are enclosed by a two-layered pericycle. There is a narrow lacuna crossed by trabeculae which consist of short endodermal cells articulating with thick-walled creeping cortical cells.

Wojinowić (23, p. 7) finds that the epidermis is provided with strongly developed hairs, which he says appear to be characteristic for the species. He says he found none on *S. Braunii*, amongst others, which he examined in this relation; but that species has well-developed trichomata, and it is by no means exceptional in this respect. Wojinowić finds two kinds of hairs on *S. lepidophylla*, one type being unicellular and the other multicellular. I have examined carefully all the material of *S. lepidophylla* I possess, and have failed to find either the one or the other; I am the more astonished at this since his paper seems to me to bear evidence of careful observation. Can he have made the mistake of imagining that the hairs on the decurrent bases of the leaves were cauline in origin? Otherwise I am forced to believe that we are working on different species. In that relation I may add that *S lepidophylla* is an easily recognized species, and that my material was derived from two sources, the Royal Gardens, Kew, where the naming is on the authority of Mr. J. G. Baker, and from the Strassburg Botanic Garden, the material in that case being named for me by Professor Dr. Kuhn, of Berlin.

20. *Selaginella helvetica*, Lk. Baker's Handbook, No. 14.

This European species has the normal arrangement of protoxylems and dorsal cords, the latter remaining for some distance distinct from the marginal strands. The epidermis is not well marked, and the hypodermis merges gradually into a thin-walled cortex whose innermost layers form a distinct reticulum with intercellular spaces. The trabeculae consist of strings of oblong or rounded cortical cells stretching across the lacuna, and articulating with short hourglass-like endodermal cells. The pericycle is one or two layers thick, and its cells contain much red colouring-matter, as do also the cells of the outer cortex. One or two layers of sieve-tubes occur dorsally and ventrally. The sieve-tubes have very long bevelled ends. Two or three layers of parenchyma separate the sieve-tubes from the xylem. The sieve-tubes are absent opposite the margins of the stele.

21. *Selaginella denticulata*, Lk. Baker's Handbook, No. 12

In anatomical characters the stem of this species approaches so closely to *S. helvetica* that a separate description is unnecessary.

22. *Selaginella pilifera*, A.Br. Baker's Handbook, No. 210.

This species is very closely related in anatomical structure to *S. lepidophylla*. The epidermis is rather more distinct than in that species. The distal cortical cells are thin-walled. The pericycle is one to two layers deep, and there is one layer of flattened sieve-tubes, absent opposite the marginal protoxylems.

23. *Selaginella patula*, Spr. Baker's Handbook, No. 52.

This species resembles closely *S. serpens* in the minute anatomy of the stem. The arrangement of protoxylems and leaf-traces is as in that species. There is little or no hypodermis. The cortex has small intercellular spaces in the layers next the lacuna. The trabeculae consist of endodermal cells articulating usually with two elongated cortical cells. The pericycle is one layer deep, though occasionally two layers occur. The phloëm is small in amount, especially on the ventral side of the stele, where not infrequently the pericycle abuts on the xylem. One layer of sieve-tubes, often interrupted, is separated normally from the xylem by one or (rarely) two layers of parenchyma (Pl. IX, Fig. 18).

24. *Selaginella convoluta*, Spr. Baker's Handbook, No. 209.

In the anatomical structure of the stem this species is near *S. lepidophylla*. There is a thick hypodermis and an inner cortex of tubular cells with small intercellular spaces. The trabeculae are of the usual type. The pericycle is one or two layers deep. One or (occasionally) two layers of sieve-tubes occur dorsally and ventrally; they are wanting, however, opposite the marginal protoxylems; they are separated from the xylem by several layers of parenchyma. The xylem is massive, and has the usual arrangement of protoxylems.

25. *Selaginella albonitens*, Spr. Baker's Handbook, No. 147.

The structure of the stem of this species is almost identical with that of *S. apus*. The sieve-tube layer passes

completely round the xylem, or is interrupted only by one or two parenchyma-cells.

The six succeeding species differ from those already described in the arrangement of the protoxylem-cords, and in the method of fusion of these. *S. flabellata* comes nearest to *S. Martensii*; the other five form their dorsal cords in a quite distinct manner.

26. *Selaginella flabellata*, Spr. Baker's Handbook, No. 245.

A section of the stele of this species shows usually three to four dorsal protoxylems in addition to the two marginal strands. These dorsal cords are formed in the usual manner, viz. by fusion of adjacent marginal protoxylems of branch and axis. The fused cords, however, remain for a long distance distinct from the marginal protoxylems of the axis beneath, although fusion between the dorsal cords themselves takes place (Pl. IX, Fig. 19). The xylem is surrounded by two or three layers of parenchyma followed by sieve-tubes, arranged two deep dorsally, one deep ventrally and opposite the dorsal protoxylems, but absent opposite the marginal protoxylems. The sieve-tubes are wider on the dorsal than on the ventral surface. The pericycle is two layers deep. The trabeculae are normal, and a small amount of silica is deposited on the inner cortical cells. The surface of at least the older stems is covered by a plentiful development of unicellular cuticularized hairs.

27. *Selaginella atroviridis*, Spr. Baker's Handbook, No. 166.

The arrangement of protoxylems in this and the succeeding four species differs considerably from that in *S. Martensii*. In the youngest branches the metaxylem is as usual not developed, its place being occupied by procambial tissue. The inner marginal protoxylem of the branch does not fuse with the adjacent marginal protoxylem of the chief axis, but runs quite distinct for a certain distance, and then fuses with the marginal protoxylem of the axis beneath, which latter is itself a downward continuation of the outer marginal protoxylem of the branch. Similarly the inner marginal protoxylem of the chief axis above the origin of the branch

runs down the stele below as a dorsal cord, which does not fuse with the marginal protoxylem of the other side until after the origin of the next older branch, when that marginal protoxylem has in turn become a dorsal cord (Pl. IX, Fig. 21).

The section of the stem is oval, and the stele is excentric in position, being nearer the ventral side. There is a cuticularized epidermis, and two to three layers of stereome. The cortex is thin-walled, and has a siliceous deposit on the inner cells. The trabeculae consist as usual of an endodermal cell and a cluster of parenchymatous cells containing chlorophyll next the cortex. The pericycle is as a rule one layer thick, though here and there two layers occur. The crushed protophloëm-elements are numerous. The sieve-tubes are two layers deep, though occasionally reduced to one layer, and are quite absent opposite the marginal protoxylems. The metaxylem is late in development, and frequently shows triradiate elements at the point of origin of branches. There are, as a rule, two layers of phloëm-parenchyma.

Dangeard (22) gives no description of the course of the protoxylems, but figures an arrangement which I have not been able to confirm.

28. *Selaginella producta*, Baker. Baker's Handbook, No. 87.

The arrangement of the protoxylems in this species is on the plan of that seen in *S. atroviridis*. The loose trabecular tissue forms a reticulum somewhat like that figured for *S. Braunii* (Pl. IX, Fig. 27). The cells composing the reticulum are frequently strongly cuticularized throughout their entire length. There is a well-marked siliceous deposit on the inner cortex and round the distal trabecular cells. The inner cortical cells are tubular, like those of *S. grandis*. The pericycle in full-grown stems is about three layers thick. The sieve-tubes are large, and two or occasionally three layers deep dorsally and ventrally, and one-layered opposite the marginal protoxylems. This layer is often interrupted in that region in smaller and younger stems. The phloëm-parenchyma is one to three layers deep.

29. *Selaginella bisulcata*, Spr. Baker's Handbook, No. 273.

The protoxylems are arranged as in *S. atroviridis*. The stele is slender and covered by a pericycle usually one layer deep. The sieve-tubes are one layer deep followed by one layer of phloëm parenchyma. There are usually two dorsal protoxylem-cords.

30. *Selaginella Bakeriana*, Bail. Baker's Handbook, No. 63.

The arrangement of protoxylems in this species is somewhat similar to that seen in *S. atroviridis*, save that the inner protoxylem of the chief axis, after becoming a dorsal cord, fuses with the outer protoxylem of the chief axis just before the origin of the next older branch of that side. Metaxylem is first developed just at the point of origin of branches where the leaf-trace of the axillary leaf is inserted (Pl. IX, Fig. 22). The pericycle is two to three layers deep. There are a few protophloëm-elements followed by the sieve-tube layer. The sieve-tubes are usually one layer deep, but here and there two and even three layers occur, especially between the dorsal protoxylems. The sieve-tubes are separated from the xylem by two to three layers of phloëm-parenchyma. The sieve-tubes are absent opposite the marginal protoxylems. The inner cortex has a small amount of silica deposited on it. (Pl. IX, Fig. 23).

31. *Selaginella concinna*, Spr. Baker's Handbook, No. 71.

The protoxylems are arranged in this species almost as in *S. Bakeriana*. There is an epidermis, slight amount of hypodermis, and a large-celled cortex. The endodermal cells articulate with the swollen distal cells of the cortex. The pericycle is two to three layers thick. The sieve-tubes are arranged in a single or occasionally double layer, but are entirely wanting opposite the marginal protoxylems. They are separated from the xylem by two to three layers of parenchyma. The cortex has a siliceous deposit on its innermost cells.

32. *Selaginella uncinata*, Spr. Baker's Handbook, No. 58.

In this species a slightly higher stage is reached in the evolution of the vascular system. An examination of a young erect shoot shows a ribbon-shaped stele with two marginal protoxylems formed by fusion of leaf-traces. At the origin of

the first branch the inner marginal protoxylems of branch and axis fuse and run down the dorsal face of the stele of the axis beneath as a dorsal cord. Fusion of this cord with the marginal protoxylem of the side on which the branch arose takes place lower down. For the first few branchings the arrangement of the protoxylems is thus of the normal type (Pl. IX, Fig. 24). Lower down, however, the dorsal cord does not fuse with the margin, but remains distinct, and presently separates away from the main xylem-ribbon as a distinct dorsal cylinder enclosed by parenchyma and sieve-tubes, but still enveloped with the main xylem-mass by a common pericycle. At the point of union of a chief and secondary axis, where the former has an isolated dorsal cord and the latter merely marginal protoxylems, fusion takes place first of all between the adjacent marginal protoxylems so as to form a new dorsal ridge, which presently separates away from the main xylem and fuses with the already isolated dorsal cord of the chief axis (Pl. IX, Fig. 25).

The stem is covered by a distinct epidermis and cuticle, three to five layers of hypodermis and a thick cortex, the cells of which latter are thin-walled and contain abundant starch. The innermost layers are composed of small cells clustered round the distal ends of the endodermal cells, with two or more of which these articulate. The pericycle is two or three layers thick. A few crushed protophloëm-elements occur here and there. The sieve-tubes are two or three layers deep and completely surround the xylem, although opposite the protoxylems only one layer occurs. Two to five layers of parenchyma separate the sieve-tubes from the xylem.

Dangeard speaks of the 'periphragme' as 'peu visible en certains points.' I have never found any difficulty in distinguishing the layers known to Dangeard by that name either in this or in any other species of *Selaginella*; and, moreover, Dangeard shows quite clearly in his figure a phloëm-area covered by a distinct one-layered pericycle. I am quite unable to interpret his scheme of the arrangement of the vascular system (22. Pl. X, Fig. 19).

B. *Oregana Type.*

33. *Selaginella oregana*, Eat. Baker's Handbook, No. 7.

This and the succeeding species are characterized by the possession of homophyllous leaves, but nevertheless a ribbon-shaped dorsiventral stele.

S. oregana resembles in habit a *Lycopodium* and is found hanging in dense tufts from N. American forest-trees in a manner similar to that shown by certain species of that genus. It possesses a ribbon-shaped stele with two marginal protoxylems. The inner cortex shows four equidistant leaf-traces, and the outer cortex four more alternate with the inner ones (Pl. IX, Fig. 30). These traces are not, however, inserted all round the stele, but on the marginal protoxylems only. The traces of the leaves which come off opposite to the margins of the stele pursue a curved course, but in the plane of the long axis of a section of the stele until, lower down, they become inserted on the marginal protoxylems. The traces of those leaves which arise dorsally and ventrally (with regard to the stele—for the stem itself is not dorsiventral) curve round in the cortex (and partly also in the pericycle) until, lower down, they in turn come opposite to the marginal protoxylems on which they are inserted. *S. oregana* thus forms a transition between the distinctly dorsiventral types, such as the majority of the monostelic Selaginellas, and the homophyllous radially symmetrical type like *S. spinosa*, where the stele, at least in its upper parts, is cylindrical and has several protoxylem areas arranged round it.

There is a large peripheral development of sclerenchyma, and the inner cortex consists of tubular cells with intercellular spaces, but without a siliceous deposit. The pericycle is from three to five layers deep. The sieve-tubes are in one or two layers and dorsiventrally flattened. They are absent opposite the marginal protoxylems, and, in the older stems, the dorsal and ventral (*re* stele) layers are frequently incomplete. One to two or more layers of parenchyma separate the sieve-tubes from the xylem.

The xylem presents the anomaly of having its metaxylem partly, at least, composed of scalariform tracheae (vessels), the transverse walls being wholly (or sometimes only partially) absorbed (Pl. XII, Fig. 111). The trabeculae are of the usual type.

34. *Selaginella rupestris*, Spr. Baker's Handbook, No. 6.

This species approaches very closely in the anatomical structure of the stem to *S. oregana*. The stele is single throughout, and has all the leaf-traces inserted on the marginal protoxylems. A dorsal cord is formed as in the typical monostelic forms, which soon fuses with the marginal protoxylem of the side on which the branch arose. The leaf-traces are very long, and those which belong to leaves not inserted opposite the margins of the stele curve round, partly in the cortex, partly in the pericycle, to be likewise inserted on the marginal protoxylems.

There is a large development of sclerenchyma which envelopes the swollen bases of the leaves; it does not, however, exist as an isolated internal annulus as figured and described by Dangeard (22). The inner cortex is small in amount and loosely arranged, trabeculae of the usual type connecting it with the stele. In any given section ten leaf-traces are seen, five within and five alternate with these outside. In Dangeard's figure seven leaf-traces are shown in the cortex, although he states, on the authority of A. Braun, that the phyllotaxis is a $\frac{5}{13}$ spiral.

The stele consists of a pericycle of three to four layers of large cells—not one, as represented by Dangeard—and one interrupted layer of very small angular sieve-tubes, quite absent opposite the marginal protoxylems. Like *S. oregana* the metaxylem consists of distinct tracheae. Dangeard's figure of the course of the leaf-traces (22. Pl. X, Fig. 4) represents nothing that I am acquainted with in the anatomy of the species.

C. *Anomalous Monostelic Species.*

I now propose to give a brief account of two species, one of which, *S. Braunii*, Baker, may be associated with species of

the *Martensii* type; the other, the well-known British species *S. spinosa*, P.B., might be associated with the homophyllous forms just described, were it not that the structure of the stele in parts of the stem is quite unlike that seen in *S. oregana*, and indeed it is quite unique in some points, so far at least as the species which I have examined are concerned.

35. *Selaginella Braunii*, Baker. Baker's Handbook, No. 236.

In this species there is a well-marked distinction between a creeping almost rhizomic axis and erect dorsiventral shoots. The chief peculiarity in the anatomy is that whilst the erect shoots are monostelic, the creeping axis is bistelic in the fully developed condition, and monostelic nearer the spore (Pl. IX, Figs. 28, 29). The stele of the erect shoot is formed from branches from both the steles of the creeping stem where there are two steles. The steles in the rhizome lie dorsally and ventrally, and are continued as distinct cords quite up to the merismatic region of the primary creeping shoot. The dorsal stele appears first as a dorsal ridge (similar to that seen so markedly in the stele of the erect shoot) on the primary stele of the creeping axis and after the origin of the first erect branch gradually separates away as a distinct dorsal stele. This stele when fully developed is broader and flatter than the ventral, and carries two marginal protoxylems (at first it has only one like the dorsal cord of *S. uncinata*). The ventral stele is concave dorsally, i. e. towards the other stele, and convex ventrally, and bears two marginal and generally two ventral protoxylems. The stele of the erect shoot is essentially a ribbon with two marginal protoxylems; one protoxylem is contributed by the dorsal, the other by the ventral rhizomic stele. These two portions run distinct for a short distance, and then fuse into the single ribbon-like stele of the erect shoot. The vascular cylinder of the root is formed in a similar manner. The stele is elliptical in section and in the youngest branches consists of two marginal protoxylems united by procambial tissue and surrounded by phloëm and pericycle. Beneath the first branch a third dorsal protoxylem appears, beneath the next a fourth. The procambium now becomes gradually transformed into

scalariform tracheids. The dorsal cords remain for a considerable distance distinct, but sooner or later fuse with the marginal protoxylems on the same side as that on which they were originally formed, after undergoing fusion amongst themselves (Pl. IX, Fig. 26).

The stem is covered by a cuticle and well-marked epidermis, the cells of which are narrow, elongated, and have thick external walls. These cells contain chlorophyll, and give rise to unicellular strongly cuticularized hairs whose almost occluded lumina are continuous with those of the epidermal cells. The hairs are longer and more numerous on young than on old stems, and the rhizome has none. The cortex consists of elongated parenchymatous cells without intercellular spaces, the cells being of greater diameter in the middle than in the inner layers. The cell-contents are scanty save in the layers next the lacuna. The chloroplastids are ovoid or rod-shaped and are accompanied by red granules. The lacuna is distinct but partially filled by a reticulum of chlorophyll-bearing cells which arise singly or in pairs from the cortex and are connected with the endodermal cells internally (Pl. IX, Fig. 27). The endodermal cells are not unfrequently branched and show the usual cuticular ring. These cells are very long at the origin of branches.

The stele is covered externally by a pericycle, usually two layers thick. A few protophloëm-elements occur just within and are followed by one to two layers of sieve-tubes. The sieve-tubes almost surround the xylem, being interrupted opposite the marginal protoxylems by one or two parenchyma-cells only. The phloëm-parenchyma occurs in one to three layers, the cells being long and narrow. Reticulate tracheids occur in the metaxylem.

Wojinowić (23) remarks that this species has no trichomata, contrasting it in that respect with *S. lepidophylla*. I have already pointed out under that species that trichomata appear to me to be wanting in *S. lepidophylla*, whilst they are most undoubtedly present in *S. Braunii*.

Dangeard (22) describes the structure of the stem of

S. Braunii (in five lines) under the name of *S. pubescens*, Spr. The present species is, however, *S. pubescens*, A. Br. *non* Spring. I feel no doubt, however, from his description that he means *S. Braunii*, Baker, and if I am right in that belief then he has failed to note the differences existing between the erect and creeping axis. He says that the species is easily recognized by the presence of trichomata; hairs, however, occur on other species, and the plant does not require such accessory aids for identification.

36. *Selaginella spinosa*, P.B. Baker's Handbook, No. 1.

It is somewhat remarkable that, notwithstanding that *S. spinosa* is one of the very few European species of *Selaginella*, and the only British representative of the genus, so little seems to be known about its anatomy. Bruchmann (18) has dealt with some points in the development of the vegetative organs, but has not apparently made any investigations into the mode of arrangement of the permanent tissues. De Bary refers to the anatomy of the stem three times in his Vergleichende Anatomie. At p. 283 (Eng. Ed.) he speaks of the stem as having 'a single axile bundle of roundish transverse section (and with a structure differing from that of other species); the leaf-bundles insert themselves on it on all sides.' At p. 343, speaking of the structure of the concentric bundle, he says that 'three (primitive groups of xylem-elements) occur near the middle in the round axial bundle in the small stem of *Selaginella spinulosa*' (=*S. spinosa*). Later on, at p. 430, he describes the sclerosis as limited to the epidermis. Dangeard (22) describes the stem as having a rounded stele with four points of protoxylem, or three by union of two of these. From these, the only anatomical references I can find, it will be seen that De Bary alone gives a suggestion of difference in the structure of the stele at different levels.

The stem is partly trailing, partly semi-erect; the leaves are throughout homophyllous and inserted all round the stem. If the stelic system be dissected out entire, it will be found that the upper part of the stem near the apex shows the leaf-traces inserted all round the stele and a series (seven in all) of distinct

protoxylem-strands can be distinguished on the outer surface of the xylem-mass (Pl. IX, Fig. 31). Lower down, however, no spiral elements are visible on the outside of the stele and the leaf-traces appear to plunge directly into the xylem. The meaning of this soon becomes apparent if serial sections be taken of the stem at different levels. At first the stele is seen to consist of a pericycle, phloëm, and central xylem-mass, having on its outer margin seven prominent protoxylem-areas. In successive sections these gradually fuse amongst themselves until only three marginal protoxylems are visible. These are, however, not prominent; on the contrary, they are more or less surrounded by metaxylem elements, save where a leaf-trace is inserted (Pl. IX, Fig. 36). Finally, in the trailing portion of the axis, the protoxylems fuse into a central patch entirely surrounded by metaxylem (Pl. IX, Fig. 37). The leaf-traces can be quite readily seen piercing the metaxylem to become united with this central protoxylem.

The stele at different levels differs entirely in detailed structure. Near the apex one finds a one-layered pericycle covering a phloëm which consists of (*a*) seven patches of sieve-tubes, one layer deep, and (*b*) one to two layers of parenchyma. Centrally there is a small-celled metaxylem of scalariform tracheides with seven patches of protoxylem alternate in position with the sieve-tube areas. In the trailing axis, on the other hand, the one-layered pericycle (which is very well marked) encloses a phloëm-tissue one to two layers deep, whose elements have exactly the appearance of the protophloëm-elements (Pl. X, Fig. 39). They possess thick, brightly refractive cell-walls, and little or no contents. The metaxylem consists of fewer and larger scalariform tracheides enclosing a patch of a dozen or so spiral and annular tracheides. Frequently the metaxylem elements abut directly on the phloëm, parenchyma-cells being here and there entirely absent. As the protoxylem becomes external, the normal sieve-tubes appear and the semi-occluded protophloëm-elements disappear. The trabeculae are normal in the erect stem, although in the creeping portion the cuticle does not separate as a distinct annulus.

The cortex of the erect axis is composed entirely of parenchyma, but in the procumbent portion one to three layers of sclerenchyma make their appearance. As Bower (27) has already pointed out, the inner cortex has well-marked intercellular spaces, and these are even more pronounced in the creeping portion.

Where the procumbent axis bifurcates, the xylem first of all becomes constricted and the central protoxylem separates into two portions, each of which becomes enclosed by the metaxylem, then by the phloëm, and finally by a one-layered pericycle.

It will be seen from the above description, and from the figures, that the anatomy of the stem of *S. spinosa* is quite different from that of the great majority of the monostelic Selaginellas, and furnishes no evidence of close relationship to such types as *S. rupestris* and *S. oregana*, near which it is placed by systematists.

D. *Galeottei Type.*

37. *Selaginella Galeottei*, Spr. Baker's Handbook, No. 185.

At the extreme apex of either a primary or secondary axis two distinct protostelic areas occur which lower down differentiate into two distinct steles. At the region between the points of origin of branches each stele possesses one protoxylem group on the outer margin (Pl. X, Fig. 41). On these the leaf-traces from the dorsal and ventral leaves of either side are inserted. At first the wood consists of protoxylem only, but about 1 cm. from the apex scalariform tracheides make their appearance and ultimately form a bulky metaxylem. In younger branchings the two steles run distinct through the 'articulation' (Pl. X, Fig. 40). The inner stele of the branch receives the axillary leaf-trace and then fuses with the inner stele of the axis, and lower down with the outer stele of the branch, running on as the marginal stele of that side of the axis beneath. Fusion of the pericycles of the steles takes place at the more apical branchings, but no metaxylem is developed until much later. In older stems the two steles of

the axis are united by metaxylem before fusion with the branch, but they separate again almost immediately.

There is an epidermis, hypodermis, and thick cortex, the last being composed of thin-walled parenchyma when young, but thick-walled and deeply pitted in older stems. The lacuna is sharply defined. The trabeculae consist of endodermal cells with or without a cluster of green parenchymatous creeping cells lying against the innermost cortex (Pl. X, Fig. 42). The pericycle consists of one layer of cells, as a rule, though two layers occur opposite the protoxylems. There is one layer of sieve-tubes, absent opposite the protoxylems on the margins of the stele, where also a few crushed protophloëm-elements occur. Two or three layers of parenchyma separate the sieve-tubes from the xylem. The swollen articulation is due entirely to hypertrophy of the middle layers of the cortex in that region, and does not affect the stele.

38. *Selaginella delicatissima*, A. Br. Baker's Handbook, No. 34.

The primary and secondary axes in this species are nearly equal, and the branching appears for that reason to be dichotomous, but falsely so. The leaf-traces of the two rows of leaves on either side fuse into a distinct stele, each lying in a wide lacuna. At the youngest branchings considerable variation occurs in the method of union of the steles (Pl. X, Fig. 43). (*a*) The steles of the branch and of the axis may both fuse in pairs, the two steles resulting from this fusion becoming the steles of the axis beneath. This is what generally happens at the first apical branching. (*b*) In the second type the same fusion in pairs occurs, but just at the points of fusion metaxylem-elements are developed so that these points are brought into close proximity, although actual fusion does not take place. (*c*) The third variation shows complete fusion of these points of metaxylem and consequent formation of a short anastomotic stele. (*d*) Lastly, and this is a method usually seen in slightly older branchings, the outer stele of the chief axis runs distinct whilst the inner stele of the axis fuses with the fused pair of steles derived from the branch. In older

conditions still the steles of the branch and of the chief axis are united by metaxylem just at the point of union of branch and axis, but the united pair belonging to the chief axis separates again into two steles before the fusion with the conjoint steles of the branch.

The stem is covered by a warty cuticle, epidermis, and, on an average, one layer of hypodermis, although this last is often wanting. The cortex consists of large delicate cells becoming smaller inwards and containing abundant chlorophyll in the layers next the lacuna. The trabeculae consist of endodermal cells articulating with loose clusters of cells lying against the inner cortex. The lacuna is large and the steles are very loosely placed in it. There is indeed, as often as not, one (more or less constricted) lacuna containing both steles. The pericycle consists of one layer of large cells. There is one layer of sieve-tubes absent opposite the marginal protoxylems and separated by one layer of large-celled phloëm-parenchyma from the xylem. The xylem is normal.

39. *Selaginella sulcata*, Spr. Baker's Handbook, No. 113.

The stems of this trailing species are bistelic between branchings, but monostelic just beyond where a branch arises. The steles of the branch unite just previous to fusion with the stele of that side of the axis on which the branch is inserted.

There is a small lumined epidermis containing red granules, a small amount of hypodermis, and a cortex, the inner layers of which are composed of cells whose walls are deeply pitted and connected at intervals with the cells belonging to the lacunar tissue. The trabeculae are simple and consist of single endodermal cells with the usual cuticular bands. The lacuna, however, contains a loose reticulum of creeping cylindrical cells arising at definite points and spreading in a radiating manner over the inner cortex (Pl. X, Fig. 44). There are no cuticular bands on these cells. The stele consists of two to three layers forming the pericyle, a few crushed protophloëm-elements, one or two layers of sieve-tubes—absent opposite the protoxylems—and two to three layers of phloëm-

parenchyma. The xylem is normal, with one marginal protoxylem on each stele.

40. *Selaginella Kraussiana*, A. Br. Baker's Handbook, No. 120.

This common garden species is one of the few that have received some attention from anatomists. Fundamentally it resembles *S. delicatissima* in structure, although it differs considerably morphologically. I have examined, in addition to *S. Kraussiana* itself, the varieties *Brownii* and *Stansfieldii*.

The mode of arrangement of the steles is quite similar to that shown in S. *delicatissima*. The epidermis is covered by a slightly warty cuticle, and is followed by one to two layers of sclerotic tissue. The cortex consists of large and delicate cells with abundant chlorophyll in the layers next the lacuna. The endodermal cells are not infrequently clustered so that two or three are embraced by one cuticular band (Pl. X, Figs. 47, 48). Some of the endodermal cells are very short, and are connected to the cortex by strings of short green parenchymatous cells (Pl. X, Fig. 46). Leclerc du Sablon (20) gives a figure of the transverse section of the stem of *S. hortensis*, a name by which *S. Kraussiana* frequently goes. This figure seems to me to be inaccurate, and his description does not help much towards clearing up the vagueness which he complains of as surrounding the question as to what are the limits of cortex and vascular cylinder in *Selaginella*. Moreover, his thesis (p. 21) cannot be maintained in view of the more recent researches of Vladescu and Strasburger.

The pericycle is one layer thick, and there is one layer of sieve-tubes, absent opposite the protoxylem. One to two layers of parenchyma separate the sieve-tubes from the xylem. The phloëm is not nearly so well developed in the primary (or older) stems. Where the steles fork at the branchings their pericycles are often connected by a reticulum of cells which show none of the characters of the ordinary trabeculae, although the cells composing the network may be partially or even completely cuticularized (Pl. X, Fig. 50).

Dangeard records an important observation with regard to

this species which, if confirmed, would tend to show that *S. spinosa* is not the only species with central protoxylem. He says: 'La tige, examinée entre la spore et les deux premières feuilles, ne présente rien de particulier en ce que concerne l'épiderme et l'écorce. Il n'en est pas de même du cylindre central; le protoxylème occupe le centre et est entouré plus ou moins régulièrement par du métaxylème; le liber entoure le tout.' This observation is of peculiar interest, seeing that Braun had already pointed out that the rhizophore of this species has also a central protoxylem. Not having as yet studied material so young, I am unable to speak on the subject from personal observation, but hope to do so later when my researches on development are completed.

41. *Selaginella Poulteri*, Veitch. Baker's Handbook, No. 193.

In anatomical structure this species very closely resembles *S. Kraussiana*. The arrangement of the steles is the same, save that the two steles between the points of origin of branches are often connected for a considerable distance by their pericycles, and even by metaxylem. Here and there the steles are separated by a reticulum of trabecular tissue, some of the cells only having cuticular annuli. Most of these cells are entirely cuticularized, whilst others again have no cuticular development, showing indeed an appearance quite similar to that figured (Pl. X, Fig. 50) as occurring at the points of origin of the branches of *S. Kraussiana*. Histologically the structure of the stele is so similar to that of *S. Kraussiana* that a separate description is unnecessary.

42. *Selaginella rubella*, Moore. Baker's Handbook, No. 183.

Anatomically this species is close to *S. Galeottei*, although it, like *S. Poulteri*, does not belong to the 'Articulatae.' A section of the erect stem, indeed, might very well be represented by Fig. 41, Pl. X, where a transverse section of the stem of *S. Galeottei* is figured. The method of fusion of steles is, however, identically the same as that in *S. sulcata*. In the procumbent parts of the stem, where the leaves are deciduous, the two steles are fused into one bulky ribbon-shaped stele,

not unlike that of *S. convoluta*, separating into two steles at the branchings. The cortex of larger stems shows an interesting anatomical feature, viz. the presence of intercellular spaces lined by siliceous deposit (Pl. X, Fig. 49). I have not been able to detect any silica in the lacuna itself.

E. *Inaequalifolia Type.*

43. *Selaginella inaequalifolia*, Spr. Baker's Handbook, No. 220.

It is one of the best known species of *Selaginella*, if for nothing else, on account of the number of times Sachs' figure of the transverse section of the stem has been made use of in botanical text-books, a figure which is, however, by no means free from inaccuracies. Dangeard (22) professes 'ressortir les relations qui existent entre la disposition des phytons et la structure de la tige.' He says that there are three steles in the stem: that entirely depends on where the section is taken; there may be as many as five, and in the primary portion of the creeping stem there is only one. The median stele Dangeard describes as having two marginal protoxylems and an anastomotic bundle: there are often two dorsal cords on the median stele. His description of the mode of origin of the steles and his figure, I have not been able to confirm. He remarks that the leaf-traces are inserted on the median stele: that is correct so far, but he adds 'exceptionellement sur l'un des cordons latéraux,' without saying which of them (the steles are, moreover, dorsal and ventral, not lateral), and without noting that the axillary leaves are invariably inserted on the ventral stele, and that no leaf-traces are ever inserted on the dorsal stele.

I have dissected out the entire stelic system of a primary shoot, after boiling in caustic potash. By this treatment the tissues are rendered clear, so that the xylem-bands may be seen with ease under a dissecting microscope. Great care in the dissection is necessary to avoid rupturing the connexion between the various steles (Pl. X, Fig. 54).

Considering first a branch axis, one finds above the first branch a single ribbon-shaped stele with two marginal protoxylems, the leaf-traces being, as in the monostelic forms, inserted on these. Where this secondary axis receives a tertiary, the mode of union of the steles is fundamentally similar to that seen in the ordinary monostelic type. The inner marginal protoxylems unite to form a dorsal cord, which fuses lower down to form with the outer marginal cord of the tertiary branch the marginal strand of that side of the axis beneath. The axillary leaf-trace inserts itself just at the junction of the inner protoxylems. Lower down, however, this strand becomes strengthened by a few tracheids from the united marginal protoxylems, and tends to form a distinct ventral cord quite similar to that formed by the united marginal protoxylems themselves. These cords presently separate from the median stele, just as in *S. uncinata*, and in section one gets three steles, the median with two protoxylems, the dorsal and ventral with one each.

If we now endeavour to trace the mode of fusion of a branch which possesses a median stele with a dorsal stele still in the condition of a dorsal cord and a ventral isolated stele, with a more vigorous axis having a ventral stele with two protoxylems, a median stele with two protoxylems and a dorsal cord like that described in *S. uncinata*, the course of fusion is found to be as follows:—The dorsal cord of the main axis fuses with that of the secondary to form a distinct dorsal stele with two protoxylems; these, however, unite with one (the outer) marginal protoxylem before fusion with the next lower dorsal cord (cf. *S. flabellata*), which in its turn forms the outer marginal protoxylem of the dorsal stele. The outer marginal strand of the median stele of the chief axis remains as the marginal strand of that side of the median stele of the axis beneath. The inner marginal strand of the median stele of the chief axis forms a connexion with, first, the inner marginal protoxylem of the ventral stele of the chief axis, but separates almost at once; secondly, with the ventral cord of the branch-axis, also separating again immediately; and,

thirdly, with the inner marginal strand of the median stele of the branch with which it runs down as an anastomotic cord for some distance, fusing ultimately with the adjacent protoxylem in the usual way. The outer marginal protoxylem of the ventral stele of the chief axis remains distinct as the outer marginal protoxylem of the ventral stele of the axis beneath; the inner strand, however, forms a short temporary union, as above stated, with the inner margin of the median stele, and then fuses with the ventral cord of the median stele of the branch axis. In short, there is a temporary union of the different steles just at the point of origin of a branch, but on that side only on which the branch arises. Finally, at the forking, three strands become inserted on the stelic system, two derived from the abortive leafy shoots (which so frequently in this species replace the rhizophores) and one from the axillary leaf. The cord from the dorsal abortive shoot is inserted on the dorsal stele, that from the ventral on the median stele, whilst the axillary leaf-trace is inserted on the ventral stele. The three primary steles may be traced at least in procambial form right up to the merismatic region in the primary shoot.

In larger and older stems the dorsal and ventral steles may each become divided, so that a small distinct stele comes to lie on the inner side of each. These steles are usually more or less united to the parent steles by pericyclar tissue. This rather complicated arrangement of the vascular cords may perhaps be made clear by a comparison of the above description with the figures.

If now the creeping shoots be examined, it will be found that all transitions are to be obtained between the tristelic condition of the erect axis and a true monostelic condition. Fig. 67, Pl. XI, shows five sections of the vascular core at different and successive levels. Fig. 67, V, represents the ordinary tristelic arrangement; in IV the ventral stele has not yet become isolated, although it exists as a distinct ventrally-placed xylem-mass; in III the dorsal stele has become almost isolated, whilst the ventral stele is merely a prominent xylem-

ridge; in II a condition similar to that seen in the adult stem of *S. uncinata* is reached; and finally in I a single, almost cylindrical, xylem-mass with four marginal protoxylems appears. Still earlier conditions can only be obtained by examination of embryonic primary shoots, material for the study of which I have not in sufficient quantity to enable me at present to illustrate thoroughly.

The histological peculiarities of the stem may next be considered. In the erect axis there is a small-celled epidermis, as usual cuticularized, several layers of stereome, and a large-celled cortex, the cells of which become smaller towards the lacunae. There are in fully developed stems three lacunae, the cortical walls of which bear a well-marked siliceous deposit. The trabeculae are short, and the endodermal cell is either connected directly with the cortical cells or with long creeping multicellular filaments enclosed in a siliceous deposit. A siliceous deposit also occurs between the double steles in old stems. The pericycle is from one to four layers thick. There are a few crushed protophloëm elements. The sieve-tubes surround the xylem, but are only one layer deep opposite the protoxylems, and may be interrupted by parenchyma opposite the protoxylems of the median stele. One to two layers of parenchyma separate the sieve-tubes from the xylem.

In the single stele of the primary creeping axis the pericycle is not markedly differentiated from the inner cortex, and cuticularized endodermal cells like those present in the erect-axis are entirely wanting. In the small intercellular spaces between the innermost layers of the cortex a minute deposit of silica appears. The sieve-tubes are quite absent opposite the marginal protoxylems (Pl. XI, Fig. 68). Where the steles separate from each other—as, for example, in situations represented at Figs. 69 and 70—the trabeculae are in the form of special cells of the pericycle, in no particular differing from those which enclose the steles, save that they are cuticularized entirely like those which form the outermost layer of the pericycles themselves. I think this is a point of some importance, as supporting the common origin of the pericycle

and endodermis, and showing that the annular cuticularization of the endodermal cells is, after all, only a special form of cuticularization following on the peculiar conditions under which these cells are developed in the mature stem.

44. *Selaginella Wallichii*, Spr. Baker's Handbook, No. 215.

The structure of the stem in this beautiful species resembles fundamentally that of *S. inaequalifolia*; the steles are, however (save in very large stems), simple, that is to say, without any accessory steles. In dissecting out the stelic system of a primary shoot, one finds a pretty constant method of fusion of leaf-traces and marginal protoxylems. Each of the simple branches has one stele; the main axis has three, which may be traced right up to the growing-point, at least in procambial form (Pl. XI, Fig. 60). The inner marginal protoxylem of the branch stele fuses with the inner marginal protoxylem of the dorsal stele, the outer fuses with the adjacent marginal protoxylem of the median axis stele. This is what one would expect on the analogy of the mode of origin of the dorsal cord of *S. uncinata*. The ventral stele is composed of the fused leaf-traces of the axillary leaves. In older branchings, where the two axes each have three steles, the mode of union is precisely that already described for *S. inaequalifolia*. The ordinary leaf-traces are inserted on the margins of the median stele only.

The primary creeping stems are at first monostelic as in the last described species.

There is a distinct cuticle and epidermis, and five to six layers of stereome, followed by an abundant thick-walled cortex, the cells of which become thinner walled inwards. The lacunae are sharply marked off. The inner cortex is covered by small elongated creeping cells, starting from definite regions. Some of these articulate with endodermal cells (Pl. X, Figs. 56–58). The pericycle is two to three layers deep, and there are many protophloëm-elements. The sieve-tubes are in two layers, dorsally and ventrally, and one (often interrupted) at the margins. One irregular layer of parenchyma separates the xylem from the sieve-tube layer. There

are often isolated parenchyma-cells scattered amongst the sieve-tubes (Pl. XI, Fig. 59). The steles (and especially the median one) are relatively broad and thin.

45. *Selaginella Willdenowii*, Baker. Baker's Handbook, No. 227.

This well-known climbing species resembles anatomically *S. inaequalifolia*. The thick leafless lower stems are tristelic, as are also the terminal primary branches; in the former the formation of accessory steles is much more common and extensive than in *S. inaequalifolia* (Pl. XI, fig. 62). From a comparison of the serial sections (Pl. XI, Fig. 61) it will be seen that there is a very close resemblance in the mode of fusion of steles in this species to that seen in the typical tristelic forms. Indeed, Fig. 61, Pl. XI, and Fig. 54, Pl. X, may be taken as, in most points, complementary to each other.

The stem is as usual covered by epidermis and cuticle. The hypodermis varies in amount from two to twenty or more layers. The cortex consists of several layers of large cells followed by smaller cells towards the lacunae, where the innermost layers are loosely arranged. The trabeculae are either simple endodermal cells, or clusters of chlorophyll-bearing cells may occur round their distal ends. The pericycle is two to four layers deep, and encloses a small amount of protophloëm. One to two layers of large lumined sieve-tubes surround the xylem, but separated from it by one to two layers of parenchyma. The sieve-tubes have both lateral and end plates. In many places, especially dorsally and ventrally, there is practically no lacuna, the pericycle being in contact with the cortex and destitute of endodermal cells.

46. *Selaginella canaliculata*, Baker. Baker's Handbook, No. 221.

Not being possessed of much material of this species, I am unable to say whether the structure of the primary shoots agrees or not with that of the branches generally. The arrangement of the vascular system, however, in the latter agrees in all essential points with that in *S. inaequali-*

folia. There is an epidermis with cuticle and a very weak hypodermis (not more than two layers thick in the material I possess), and a large-celled thin-walled cortex, the cells of which become smaller towards the lacunae. There are three lacunae, each containing one stele with marginal protoxylems. The lacunar tissue shows an interesting modification (Pl. XI, Fig. 63). The endodermal cells are stout and long, and run direct from the pericycle to the cortex. The lacunar space is, however, filled by a loosely-arranged succulent parenchyma of rounded chlorophyll-bearing cells, having no connexion with the endodermal cells.

The pericycle is one to three layers thick, and has a prominent cuticle. Protophloëm-elements occur here and there, especially opposite the marginal protoxylems. The sieve-tubes pass quite round the xylem—one layer deep opposite the protoxylems, and two layers deep elsewhere. The sieve-tube layer is imperfect or wanting opposite the marginal protoxylems of the median stele. One or two layers of phloëm-parenchyma separate the sieve-tubes from the xylem.

47. *Selaginella Mettenii*, A. Br. Baker's Handbook, No. 102.

This species is believed by some systematists to be a hybrid between *S. inaequalifolia* and *S. uncinata*. The anatomy of these two species, so far at least as the stem is concerned, is sufficiently distinct, as has already been shown, and one would expect to find intermediate characters in the hybrid, if hybrid it be. I cannot say I have found such in the stem of *S. Mettenii*. The stem is regularly tristelic, and the course and mode of fusion of the steles is precisely that already described in the case of *S. inaequalifolia* (Pl. XI, Fig. 64). The median stele is strap-shaped, and bears two marginal protoxylems; the dorsal and ventral steles are rounded, and generally, when small, carry one protoxylem each (Pl. XI, Fig. 65). The epidermis and cuticle are succeeded by two to three layers of stereome. The cortex is large-celled, the cells as usual becoming smaller towards the lacunae. The trabeculae consist of endodermal cells, with or without clusters of green

parenchymatous cells round their cortical ends. The pericycle is one to two layers deep. There is one layer of sieve-tubes (absent opposite the marginal protoxylems of the median stele), and separated from the xylem by one or two layers of parenchyma.

48. *Selaginella Lobbii*, Moore. Baker's Handbook, No. 217.

The arrangement of the protoxylem-strands and anastomosis of the steles at the branchings is precisely similar in this species to that seen in *S. inaequalifolia*. There is an epidermis, cuticle, feeble hypodermis and thick cortex ending abruptly at the lacunae, where there is a slight siliceous deposit. In the erect primary shoot there are three chief steles, with accessory steles (in thick stems) as in *S. inaequalifolia*. The trabeculae consist of simple endodermal cells, but the lacunae are more or less filled with green parenchymatous cells, which arise in clusters from the inner cortex. The pericycle is, in the fully developed stele, about three layers thick. The sieve-tubes are in two layers, or occasionally even three layers occur, save opposite the protoxylems, where they occur in a single layer. In the median stele the sieve-tube layer is interrupted opposite the marginal protoxylems. A few crushed protophloëm-elements are seen beneath the pericycle, and one layer of parenchyma separates the sieve-tubes from the xylem (Pl. XI, Fig. 74).

In the primary creeping stem a condition of things similar to that seen in *S. inaequalifolia* appears. In the earliest conditions that I have been able to examine there is a four-rayed xylem-mass with four points of insertion of leaf-traces (Pl. XI, Fig. 66 iv). The protoxylem elements are partially sunk in the metaxylem. Possibly earlier conditions still may show appearances recalling the central protoxylem of *S. spinosa*; but I am unable to give further details until I have studied the phenomena of germination in the species. The two ventrally placed bands soon separate from the main xylem-mass, though they are still united by a common pericycle. The dorsal cord then becomes isolated, and the tristelic condition of the erect stems is reached.

49. *Selaginella gracilis*, Moore. Baker's Handbook, No. 216.

This species does not differ anatomically from the typical tristelic form, save that in older branchings the dorsal stele of the chief axis below the origin of a branch is formed by the fusion of the dorsal steles of the chief axis and of the lateral branch, the median steles of the primary and secondary axes behaving like the single steles of the primary and secondary axes of the typical monostelic forms. There is, as usual, a fusion of all the steles just at the point of insertion of the axillary leaf.

In the creeping stems a gradual fusion of steles occurs, as in other tristelic species, the earliest condition being monostelic.

50. *Selaginella viridangula*, Spr. Baker's Handbook, No. 224.

The chief anatomical characters of the stem of this species are the slight fusion that occurs between the various steles at the points of origin of branches, and the tendency which the ventral stele has to split into two parallel steles. The course of the steles is as in *S. gracilis*. There are no special histological characters which distinguish *S. viridangula* from the normal tristelic forms.

51. *Selaginella chilensis*, Spr. Baker's Handbook, No. 225.

This species is said to be possibly conspecific with *S. canaliculata*, Spr. Anatomically and histologically a description of the stem of the one might stand for that of the other; it is therefore unnecessary to repeat the account already given for that species.

52. *Selaginella Victoriae*, Moore. Baker's Handbook, No. 218.

The creeping axis of *S. Victoriae* shows a transition from a monostelic to a tristelic condition entirely similar to that seen in *S. inaequalifolia* (Pl. XI, Fig. 67). In the erect shoots, which are also tristelic, the ventral stele generally bifurcates, as in *S. viridangula* (Pl. XI, Fig. 72). The structure of the steles, and their mode of origin and fusion, agrees fundamentally with the other tristelic species already described.

F. *Lyallii Type.*

The solitary form which I describe in this section is considered by Baker as a variety of *S. laevigata*, Baker. I have made several attempts to obtain *S. laevigata* itself in a fresh state, but without success. Under these circumstances I have thought it best to designate the section by the name of the variety, for it does not follow, as I have shown above, that outward resemblance is accompanied by identity or even similarity of internal structure.

53. *Selaginella laevigata*, Baker; var. *Lyallii*, Spr. Baker's Handbook, No. 251.

Baker describes this plant as having 'Stems erect, 1–1½ feet long, simple in lower half, the leaves small, distant, and soon deciduous, deltoid in the upper half, with petioled deltoid 1–2 pinnate pinnae.' He makes no mention in the diagnosis of the marked distinction to be seen between the erect stems and the short zigzag rhizome. Spring speaks of the stem as 'e basi repente radicante erectus.' The distinction between creeping and erect stem is emphasized by the marked anatomical differences between those two portions of the axis. Indeed, the differences are so great that the two parts might well belong to different genera. The stem has again and again been referred to, though briefly, in anatomical works, but the descriptions apply to the erect stems only, the creeping stem not even being mentioned (Pl. XII, Figs. 77 and 79).

The anatomy of the stem as a whole will perhaps be most readily understood by commencing with a consideration of the branch system.

1. *Anatomy of a branch-system.*

If the stelic system of a terminal pinna be isolated in the usual manner, it will be found (Pl. XII, Fig. 78) that there are, to start with, two distinct steles, each with pericycle, endodermis, and lacunar tissue. Each has a phloëm-area and one protoxylem-strand only, derived from the fused leaf-traces of the leaves of the dorsal and ventral leaves. Very soon, and before the fusion with the stelic system of the first branch, the

stele of that side on which the branch arises splits into two steles, one carrying the traces of the dorsal leaves of that side, the other carrying the traces of the ventral leaves of the same side. A similar splitting takes place in the other stele on the side away from the origin of the branch. There are thus differentiated, above the origin of the youngest branch, the four steles which form the basis of the vascular system. The steles are generally connected in pairs by their pericycles, or at least occupy a common lacuna. When the branch fuses with the more vigorous axis, the stele of the branch which lies next to the chief axis fuses with the dorsal stele of the pair on that side, whilst the outer stele of the branch fuses with the ventral stele of the main axis of the same side, which latter receives also the leaf-trace of the axillary leaf. The two conjoint steles now pass downwards but fuse, at least so far as their pericycles are concerned; so that a section of a slightly older axis may show only two steles laterally placed; or three, where the right or the left pair may be connected by their pericycles; or four, when all are distinct as in more mature branches.

2. *Anatomy of the primary erect shoot.*.

The unbranched portion of the erect shoot shows in section eleven, twelve or even thirteen steles, though two or more of these may be united by their pericycles. The accessory steles are separated off from the four primary steles (i. e. from those which carry the leaf-traces), apparently without any very definite order; for after examining several shoots I found so much variation in the branching of the accessory steles, that I felt that the number and order of these could not be a point of fundamental importance.

If the vascular system of an erect shoot, still unbranched, be traced from the apex down to the creeping rhizome, it will be found that quite at the apex the four primary steles are clearly distinguishable in procambial form—and alone receive the leaf-traces; within are to be seen (in a definite instance) seven accessory steles, one of them double. These accessory steles do not carry leaf-traces, but separate away from, or rather anastomose with the four primary steles lower down.

By taking serial sections it may be seen that a gradual fusion amongst the steles of the erect axis takes place as the creeping stem is approached, until there is left finally one central cord and four steles (which bear the leaf-traces), regularly arranged round it. These latter then fuse, first in two pairs, and finally just above the origin of the shoot from the creeping axis, into one semicircular stele, with the central stele lying distinct in the convexity. Figs. 83 to 89, Pl. XII, illustrate the gradual fusion amongst the steles of the erect shoot as the rhizome is approached.

3. *Anatomy of the creeping axis.*

If now the creeping axis be examined a totally different arrangement of vascular tissue is disclosed. The creeping stem, like the erect unbranched axis, has homophyllous leaves, but these are still arranged in four rows. The leaves are closely packed and the growing region is very short. If a transverse section be made midway between the points of origin of any two erect shoots, it will be seen that the centre of the section is occupied by a stele whose xylem has no protoxylem elements, and which is surrounded by an ill-defined lacuna. This stele is enclosed completely by another cylindrical stele consisting of pericycle, phloëm, and a well-marked ring of tracheids, with four protoxylem-areas (or three by fusion of two of these) on its outer edge, to which the leaf-traces are attached. Just before the erect shoot arises, the outer cylindrical stele opens, becoming horse-shoe shaped in section, the opening being on that side on which the erect shoot originates, and a fusion takes place between the central stele and the upper wing of the crescentic outer stele. If the entire stelic system of the erect shoot and creeping axis be isolated and compared with serial sections, it will be found that the crescentic band of the erect shoot fuses with the cylindrical stele only, and that the central cord of the erect shoot fuses chiefly with the central stele, but is connected with the external cylinder as well. Towards the growing-point of the creeping axis the outer stele gradually becomes narrower, and finally ends in merismatic tissue. So

far as I have been able to determine at present, the central cord may be traced also nearly up to the region where tracheids first make their appearance in the external cylindrical stele, but there it approaches and seems to be separated off from the inner border of the cylindrical stele. Here and there also, in the rhizome generally, the central cord fuses with the external stele, and the centre is occupied by parenchyma (Pl. XII, Fig. 93).

4. *Structure of the stele and cortex.*

A distinct epidermis covers the stem, the outer walls of the epidermal cells being lamellated. There is a well-marked cuticle. The hypodermis consists of from eight to twelve layers of thick-walled sclerenchymatous cells, not nearly so abundant, however, in the rhizome. The cells of the general cortex are thick-walled and show a peculiar condition of the middle lamella. At the angles of the cells the lamella forms a triradiate figure, but the secondary thickening does not come quite up to the corners, so that between the cells there are minute tubular spaces divided by the lamella into three triangular spaces (Pl. XII, Fig. 80).

Next the lacuna the cells are smaller and loosely arranged. The lacunae, which are well defined, are occupied by trabeculae, some of which consist simply of an endodermal cell with the usual cuticular band, whilst others are connected on the cortical side with creeping chlorophyllaceous cells over the inner side of the compact cortex (Pl. XII, Fig. 81).

The structure of all the steles of the erect shoot is fundamentally the same. There is externally a cuticularized pericycle one or two layers deep, the cells of which, as usual, contain starch, chlorophyll, &c. Within the pericycle several patches of crushed protophloëm-elements occur enclosing in turn a layer of sieve-tubes, separated from the xylem by one or two layers of parenchyma. The sieve-tubes almost surround the xylem and actually do so in the accessory steles. Many of the accessory steles show two protoxylem-areas, but the primary steles one only, which is turned outwards to receive the leaf-traces.

As already described, the structure of the rhizome is very different from that of the erect axis. The large-celled cortex towards the stele becomes smaller-celled and almost, save for a few small intercellular spaces, continuous with the outer pericycle layers of the cylindrical stele. (Compare the creeping axis of *S. inaequalifolia*, p. 181.) In these spaces occur cells which are homologous with the endodermal cells, but whose walls do not show the usual cuticular band. On the other hand, their walls are wholly cuticularized and they stand out quite prominently in sections as bright clear rings. There follows, passing inwards, several layers of small-celled pericycle, and then an interrupted layer of small tangentially flattened sieve-tubes. The sieve-tubes are separated from the xylem by (on an average) two layers of phloëm-parenchyma, quite similar to and, in section, about the same size as the cells of the pericycle. The ring of xylem is quite complete (save where, as already explained, the erect shoots arise) and is on an average two tracheides broad. On the outer side and alternating with the patches of small sieve-tubes lie four, or near the origin of erect shoots, several protoxylem-areas. Within the tracheidal ring one meets with another double layer of phloëm-parenchyma, followed by one or usually two layers of large sieve-tubes, continuous round the inner side of the stele. These are followed by a pericycle similar to that on the outside and by endodermal cells wholly cuticularized and lying in an irregular and not well-marked lacuna and connecting the pericyles of the inner and the outer steles. The structure of the inner stele is somewhat different. There is a pericycle about two layers deep and a single, or occasionally double, layer of sieve-tubes completely enclosing a single layer of parenchyma which in turn surrounds a central mass of xylem consisting of about a dozen scalariform tracheids without any protoxylem-elements. As already mentioned, this central stele may be absent as a distinct vascular strand, appearing merely as a ridge on the inner border of the outer and cylindrical stele. It is perhaps worth while recalling in this connexion the structure of such a form

as *Lepidodendron Harcourtii*, With., where also, according to Williamson's researches (*Phil. Trans.*, 1893), there exists a central cylindrical stele enclosing a medulla and bearing protoxylem-areas on the outer margin of the ring of metaxylem. I have looked in vain for any evidence of secondary increase of the xylem in the material I possess of *S. Lyallii*, but am hopeful of obtaining ere long much older rhizomes from Madagascar which, it is quite possible, may afford some evidence of a closer relationship between the modern Selaginellas and the ancient Lepidodendra than is at present forthcoming. I am not aware that any published account has hitherto appeared of the existence of such a condition of the stele in the Selaginellaceae as that I have described above, and it seems to me that the results arrived at ought to have an important bearing on the study of the phylogeny of the genus.

Dangeard's description of the structure of the stem of *S. Lyallii* seems to me very inadequate. He has apparently never dissected out the stelic system, nor has he even suspected a fundamental difference in structure in the rhizome. Moreover, he takes the arrangement of steles in the branch as equivalent to that of a primary shoot, and entirely omits any mention of detailed histology.

Section II. Comparative Summary.

Dangeard alone has ventured to give a comparative summary of the anatomy of the genus. It will be convenient for future reference to outline his views at this point. After a preliminary summary of the histology which I have criticized in the historical introduction to this paper, the author goes on to distinguish certain anatomical types as follows:—

1. The stem possesses four foliar cords, isolated and separate. Under this section Dangeard places such forms as *S. uliginosa* (a species I have been unable to obtain), *S. spinosa*, and *S. rupestris*. I have pointed out under the two latter species that his description of these is both inaccu-

rate and incomplete. He also groups with this series *S. Lyallii*, that species having the peculiarity of possessing anastomotic cords as well. In the first place, I differ entirely from him in admitting the legitimacy of the comparison which he has thus instituted between an erect secondary shoot of *S. Lyallii*, and the trailing stem and its erect continuation in *S. spinosa*. Further, he seems to have failed to recognize that in *S. Lyallii* there are four foliar cords from the very beginning in the erect shoot, each enclosed in its own pericycle and endodermis, whilst in *S. spinosa* there is only one stele with several protoxylem-regions. He remarks that the bundles are arranged without order in the stem of *S. Lyallii* (22, p 245, 'et se dispersent sans ordre dans les tiges'); that I cannot agree with, and moreover, De Bary had clearly stated long previously that the arrangement of the cords was such as to form 'three equidistant rows in an almost quadratic surface' (16, p. 283).

2. The stem possesses two foliar cords.

A. These are united by metaxylem into one median stele. This, Dangeard remarks, is the most frequent arrangement, and is due to the fusion of the four original cords in pairs. He also remarks on the presence of an anastomotic cord in such species.

B. The two foliar cords may remain isolated. This arrangement is found, he says, in the articulate species, where, however, the cords fuse, as in A, at the points of origin of branches. It is necessary to point out that other species than those belonging to the so-called 'Articulatae' show this mode of arrangement.

3. The stem possesses three parallel vascular bands. In this case Dangeard distinguishes (*a*) the presence of a median stele similar to that occurring in 2. A, and (*b*) two lateral (*sic*) vascular strands isolated from the median stele at the bases of the ramifications. These may become fragmented in various ways, as in *S. laevigata* (= *S. Willdenowii*, Baker, non *S. laevigata*, Baker). They bear several protoxylem-strands, and exceptionally receive leaf-traces. I have already criticized

this statement, and pointed out how in *S. inaequalifolia* these dorsal and ventral accessory steles arise.

It will have been seen, from the data given in some detail in the first section of this paper, that several distinct types of stem-structure may be distinguished. I propose now to give a brief summary of these types, arranged in the order of what seems to me their phylogenetic development.

1. *S. laevigata*, Baker, var. *Lyallii*, Spr.

In this form a distinct rhizome gives origin to a series of erect shoots on the one side and roots on the other. The rhizome contains a cylindrical hollow stele, with protoxylems on the outer border of the xylem. The centre of the cylinder is occupied by parenchyma, or by a cord of metaxylem without protoxylem-elements, derived from the inner margin of the cylindrical stele. The cylinder opens opposite the point of origin of each of the erect shoots, and the steles of the erect shoot are inserted on the upper (dorsal) border of the mesh, and are connected also with the central cord, which in this region fuses with the cylindrical stele. The erect shoots possess four primary cords, on which the leaf-traces are inserted, and several accessory cords which anastomose with each other and with the primaries. The ultimate branchlets are tristelic, as in the normal tristelic species. At the union of such a branchlet and the more vigorous axis possessed of four cords in two lateral pairs, the members of which are dorsal and ventral, the inner stele of the branch fuses with the dorsal stele of the pair of that side, the outer with the ventral. The trace of the axillary leaf is inserted on the ventral stele.

2. *S. spinosa*, P.B.

In this type there is no marked distinction between procumbent and erect axes; the stem is at first creeping and then becomes erect or semi-erect. The creeping portion, however, shows the anomaly of having a central protoxylem. The erect portion of the shoot may be compared with the creeping rhizome of *S. Lyallii*, for there also there occurs a single cylindrical stele with protoxylems on the outer border of the metaxylem, which are the points of insertion of the

leaf-traces. The leaf-traces on the region of the stem which has a stele with central protoxylem pierce the metaxylem and fuse with the central protoxylem cord. The cylinder is, however, not hollow, although in the apical region the metaxylem is preceded by procambial tissue.

3. *S. Galeottei*, Spr.

The so-called bistelic species may be derived from stems like those of *S. Lyallii* (where the leaves are arranged in four rows), by fusion of the protoxylems of adjacent leaf-traces and feeble development of metaxylem, so that two laterally placed steles result each with one protoxylem strand marginally situated. The steles of the chief axis fuse at or near the origin of branches, and the two steles of the branch unite before their insertion on the stele of that side of the main axis.

4. *S. Braunii*, Baker.

In this type the creeping axis is differentiated from the erect shoots, as in *S. Lyallii*, and is at first monostelic. Later it becomes bistelic, the steles being dorsal and ventral, not lateral. The erect shoots are, however, monostelic, the two marginal protoxylems arising from the dorsal and ventral steles respectively.

5. *S. oregana*, Eat.

In this species we have a transition between *S. spinosa* and the usual *Selaginella* type; for here, although the leaves are homophyllous, the stele is dorsi-ventral, and consists of a ribbon with two marginal protoxylems and one dorsal protoxylem formed by fusion of the adjacent marginal protoxylems of branch and axis.

6. *S. Martensii*, Spr.

Round this species may be grouped the majority of the species of the genus, diverse as they are in habit, all characterized by dorsiventrality both of external morphological features and internal anatomy, and by the possession of a single stele.

7. *S. uncinata*, Spr.

This type is particularly interesting, inasmuch as it gives the first indication of the tendency to form at least three distinct steles placed dorsally, medianly, and ventrally, and

found in the highest division of all, arising, as I have tried to show, in quite another way from the bistely of such forms as *S. Galeottei.* The semi-distinct dorsal cord arises in *S. uncinata* in the same way as the dorsal protoxylem-ridge of such a type as *S. oregana* or *S. Martensii*, only in *S. uncinata* it becomes more robust and is more or less dissociated from the main xylem-mass.

8. *S. inaequalifolia*, Spr.

I look upon the series, of which this species may be taken as the type, as representing the highest and most specialized development of the stem in the genus. Here two principal accessory steles have been formed, one dorsal to the median stele, which alone bears the ordinary leaf-traces, the other ventral. The dorsal stele arises by fusion of cords which, in younger conditions, form the adjacent marginal protoxylems of branch and axis, whilst the ventral arises from a fusion of the leaf-traces of the axillary leaves, strengthened by elements derived from the median stele, where fusion takes place at the points of origin of branches.

Comparative histology.

The superficial layer of the stem, to which for convenience of description the name of epidermis may be applied, is invariably covered by a more or less well-developed cuticle, which is in some cases (e.g. *S. delicatissima*, *S. Kraussiana*) provided with minute warts similar to those which occur so frequently on the cuticle of the leaves. The epidermal cells are elongated, and have generally thick lamellated walls, especially on the outer aspect. They may, as in *S. apus*, *S. Douglasii*, *S. molliceps*, &c., be scarcely distinct in character from the hypodermal cells beneath, but more commonly they form a well-marked and distinct layer, e.g. *S. haematodes*, *S. involvens*, *S. lepidophylla*, &c. They may be uniform in character throughout, i.e. thin-walled and elongated, or thick-walled and lignified; or elongated and thin-walled on the dorsal and ventral surfaces of the stem, and short, thick-walled, and deeply pitted near the bases of leaves. The epidermal cells contain chlorophyll and occasionally red colouring-matter.

Unicellular cuticularized hairs, whose cavities are continuous with those of the epidermal cells, occur in a few species, e. g. *S. Braunii*, *S. flabellata*, *S. Vogelii*, &c. In such cases the erect shoots alone bear hairs.

The erect stems are strengthened by the development of a sclerotic hypodermis which gradually merges into the thinner walled general cortex. The cells are much elongated and taper at either end. Their walls are thick, and as a rule, deeply pitted and give the lignin reaction. Stereome is developed chiefly in the erect shoots, and not infrequently is entirely absent from the rhizomic parts of the axis. In *S. haematodes*, and other forms, the walls have a bright red colour. In many, e. g. *S. apus*, *S. molliceps*, &c., the stereome is much reduced, never being more than one to two layers deep; in *S. spinosa* (erect axes) the sclerosis is limited to the epidermal layer. In others, again, e. g. *S. involvens*, *S. lepidophylla*, &c., there may be twenty or more layers of stereome. The hypodermal cells may contain chlorophyll, though in small amount. In cases where the bases of the leaves are swollen, e g. *S. rupestris*, sclerotic tissue may be developed round and on the leaf bases. Intercellular spaces are entirely absent from this region. The cortex proper varies much in amount, and merges gradually on the outer aspect into the peripheral stereome, and inwardly into the lacunar tissue. The cells are as a rule long and end abruptly, not tapering as in the case of the hypodermal cells. They are large and delicate in some forms, such as *S. Kraussiana*, *S. delicatissima*, &c., or thick-walled and pitted as in *S. grandis*. The cells are invariably narrower as the lacuna is approached. In many species the inner cortical cells are narrow and tubular, and very loosely arranged so that intercellular spaces are by no means infrequent. Occasionally, as in *S. haematodes*, the inner cortical cells are sclerotic, and very many species show a deposit, more or less abundant, of silica both in the intercellular spaces and on the surface-layer facing the lacuna. In *S. involvens* intercellular spaces occur quite up to the commencement of the hypodermis. Intercellular spaces lined with silica occur in the outer cortex of *S. rubella*. The

peculiar condition of the middle lamella in the thick-walled cortex of *S. Lyallii* has already been described (p. 190). Chlorophyll and starch are most abundant, as a rule, in the inner layers of the cortex, and these vary in amount from only a few grains to a very large quantity, as in *S. viticulosa.* As already explained, the so-called 'articulations' of the *Articulatae* are due entirely to hypertrophy of the middle layers of the cortex.

The trabecular tissue, under which may be included both the endodermal cells and the parenchymatous tissue associated with and connecting these to the more compact cortex, varies very considerably in character. The two commonest conditions found are, (*a*) an endodermal cell articulating with two swollen chlorophyll-bearing cells, which in turn are attached to the cells of the inner cortex; and (*b*) a similar condition, only the distal parenchymatous cells undergo division and form a cluster of cells surrounding the distal end of the endodermal cell. The endodermal cell is most commonly a longer or shorter tubular cell, containing protoplasm and a nucleus but no chlorophyll, arising from the pericycle, and possessing, usually, a well-marked cuticular ring medianly placed. In older conditions the cuticularization may spread over the entire wall, and in other cases still, e. g. procumbent axis of *S. spinosa*, rhizome of *S. Lyallii*, &c., the whole wall is uniformly cuticularized from a very early stage in the development of the cell. In some cases two or more endodermal cells may be found enclosed in a common cuticular band, the cells in such cases never having become isolated. In *S. Braunii* the endodermal cells are not infrequently branched. In other cases still, e. g. *S. canaliculata*, the endodermal cells run across the lacuna, articulating directly with the more compact cortex.

The special distal cortical cells, to which in most cases the endodermal cells are attached, are most commonly swollen and succulent and contain a large amount of chlorophyll. Very frequently these cells undergo considerable subdivision, so that a fairly large amount of parenchyma is found surrounding the distal end of the endodermal cell. In other cases these cells may be long and tubular, and more like those of the inner com-

pact cortex, e.g. *S. grandis*. In *S. viticulosa* the endodermal cell articulates with two to five or even more long twisted cells packed with large chlorophyll-bodies and starch, and not infrequently clusters of similar cells arise from the inner cortex and partially fill the lacuna, but are quite disconnected from the endodermal cells (e.g. *S. sulcata*). In many species the endodermal cell articulates with one intermediate distal cell or with a single row of such (*S. helvetica*). In *S. Braunii*, *S. Poulteri*, &c. a reticulum of cells which cannot be distinctly recognized as genuine endodermal cells or inner cortical tissue, separates the stele from and at the same time connects it with the cortex or with another stele. Rows of cells, which are identical in character with those of the pericycle, connect the steles of such forms as *S. inaequalifolia*. Finally, the lacuna may be entirely filled with tolerably compact parenchymatous tissue through which the endodermal cells run (*S. canaliculata*), whilst in the creeping axes of several species no distinct trabecular tissue is developed at all, the pericycle being in such cases continuous with the general cortex. It will be seen, therefore, that the well-marked lacuna and specialized trabecular tissue of the erect shoots of most species is a quite peculiar adaptation easily accounted for and traceable to a much more generalized development of intercellular spaces not at all uncommon in the Lycopodinae (cf. 27). The pericycle invariably consists of one or more (four to five being a maximum) layers of long thin-walled cells containing chlorophyll (if the axis be above ground), the wall next the lacuna being covered with a more or less well-marked cuticle. The pericycle is always perfectly distinct and easily recognizable.

The protophloëm-elements are delicate, tapering, and semi-occluded, occasionally showing a fairly well-marked pitting on their walls, and containing, when they exhibit any cavities, a small amount of granular proteid substance. The walls are very irregular in thickness but always highly refractile. They may be best seen, perhaps, in *S. Wallichii*, where they are very numerous; in other cases they are few in number, or entirely absent.

The sieve-tubes have already been carefully described by Janczewski (17), and I have been able to confirm his account in every species in which I have paid special attention to these elements. Their walls are exceedingly delicate and show numerous but difficultly recognizable sieve-plates on such walls as abut on other sieve-tubes. Both lateral and end plates occur; and it is possible to get a callus reaction from the thickenings which are not infrequent on the plates of large and old stems, e.g. *S. Lobbi* and *S. Willdenowii*. The phloëm-parenchyma, which forms a well-marked layer between the sieve-tubes and the xylem, consists of much elongated richly protoplasmic cells from one to four or more layers deep. Not infrequently isolated cells or groups of such are found amongst the sieve-tubes themselves. The distribution of these cells in relation to the sieve-tubes has already been described. The protoxylem-elements are tracheides and are spiral, annular or intermediate in character. The spiral elements may have one, two or three spiral thickenings, and considerable variety in the arrangement of the threads may be found. Save in two cases, the metaxylem consists entirely of scalariform or reticulate tracheides, the former being by far the more common. Occasionally the tracheides are branched, especially near the origin of branches. In *S. oregana* and *S. rupestris*, however, the tracheidal character is lost, and the elements are distinctly tracheae or cell-fusions partial or complete. I believe that this feature, though occurring but rarely in true ferns, has not hitherto been shown to occur in the Lycopodinae. I am not prepared to say that these species are exceptional in this respect, but so far as I have examined they appear to be so.

In concluding the present paper, I would like to call attention to the fact that, taking into account the anatomy of the stem only, we have been led to group together species widely separated by systematists, and conversely to separate species which agree strongly in external characters. Even in such

a group as the *Sarmentosae* section of the subgenus *Stachygynandrum*, which so nearly agrees with the anatomical section of which *S. inaequalifolia* is the type, we find *S. Mettenii* widely separated from the type and *S. Willdenowii* refused a place. A more prominent case, however, is that of *S. Lyallii*, which, although it has a quite unique anatomical structure, stands side by side with species like *S. Vogelii* and *S. grandis*, forms not separable histologically from *S. Martensii*. How far comparative anatomy may serve as a basis for a revision of the established classification of the Selaginellaceae, and how far it supports or otherwise external morphology, can only be determined after extended observation on all the members and not the stem only. Meantime I think it worth while to draw attention to the fact that anatomy does not support the classification based on external morphology, at all events so far as the stem-structure of the above-mentioned species is concerned.

EXPLANATION OF FIGURES IN PLATES IX, X, XI, AND XII.

Illustrating Prof. Harvey Gibson's paper on the Anatomy of *Selaginella*.

(The magnification employed is indicated in each case, but the figures in some cases have for convenience been reduced).

PLATE IX.

Figs. 1–5. *S. Martensii*, Spr.

Fig. 1. Dissection of the stele of a primary shoot after treatment with *KOH*, viewed from the dorsal surface. The axillary leaf-trace is indicated by an arrow-head. *a*, the dorsal cord formed by fusion of the adjacent marginal protoxylems of branch and chief-axis (× 10).

Fig. 2. A small portion of the stele in transverse section. *a*, pericycle; *b*, sieve-tubes; *c*, phloëm-parenchyma; *d*, protophloëm-elements; *e*, metaxylem-elements (× 550).

Fig. 3. *a*, *b*, *c*, successive stages in the development of the cuticularized endodermal cell, showing mode of origin of the annulus (× 800).

Fig. 4. Bifurcate endodermal cell from the point of origin of a branch (× 800).

Fig. 5. Cuticular annulus seen from above, *a*, before, *b*, after isolation of the ring from the pericyclar cuticle (× 800).

Figs. 6–7. *S. grandis*, Moore.

Fig. 6. Transverse section of the median part of the stele. *a*, *a*, pericycle ; *b*, *b*, sieve-tubes; *c*, *c*, phloëm-parenchyma ; centrally, metaxylem (× 800).

Fig. 7. Lacuna and trabecular tissue (× 800).

Fig. 8. *S. Vogelii*, Spr.

Longitudinal section through the pericycle and trabecular tissue (× 800).

Fig. 9. *S. haematodes*, Spr.

Transverse section through the pericycle, trabecular tissue and inner cortex; the dark shading indicates siliceous deposit (× 800).

Fig. 10. *S. erythropus*, Spr.

Strongly sclerotised fibres of the outer cortex of the creeping axis in transverse section (× 550).

Fig. 11. *S. Griffithii*, Spr.

Transverse section of the margin of the stele, showing a single layer of sieve-tubes surrounding the protoxylem (× 800).

Figs. 12–13. *S. viticulosa*, Kl.

Fig. 12. Longitudinal section of the stem showing twisted trabecular cells (× 550).

Fig. 13. Chloroplastids from the trabecular cells (× 800).

Figs. 14–15. *S. serpens*, Spr.

Fig. 14. Transverse section of the stem (semi-diagrammatic). *a*, hypodermis; *b*, inner cortex ; *c*, *c*, leaf-traces of dorsal leaves ; *d*, *d*, leaf-traces of ventral leaves ; *e*, lacuna round leaf-trace ; *f*, lacuna round stele ; *g*, pericycle ; *h*, sieve-tube layer ; *i*, phloëm-parenchyma ; *k*, metaxylem ; *l*, marginal protoxylem ; *m*, dorsal cord (× 30).

Fig. 15. Trabecula from the origin of a branch (× 550).

Fig. 16. *S. molliceps*, Spr.

Transverse section of the stele showing irregularity of the pericycle and small amount of phloëm (× 550).

Fig. 17. *S. involvens*, Spr.

Portion of the inner cortex in section. The dark shading indicates SiO_2 (× 550).

Fig. 18. *S. patula*, Spr.

Transverse section of a small portion of the stele and inner cortex (× 550).

Figs. 19–20. *S. flabellata*, Spr.

Fig. 19. Portion of the stele dissected out and viewed from the dorsal side, showing the arrangement of the dorsal cords (× 10).

Fig. 20. The stele in transverse section ; the irregular dark outer line represents the siliceous deposit on the inner cortex. *a*, pericycle; *b*, sieve-tube layer; *c*, phloëm-parenchyma ; *d*, one of the dorsal protoxylems (× 30).

Fig. 21. *S. atroviridis*, Spr.

Scheme of arrangement of protoxylems in the stele (× 10).

Figs. 22–23. *S. Bakeriana*, Bail.

Fig. 22. Scheme of arrangement of protoxylems (× 10).

Fig. 23. A trabecula; the dark shading indicates siliceous deposit (× 550).

Figs. 24–25. *S. uncinata*, Spr.

Fig. 24. Scheme of the arrangement of protoxylems (× 10).

Fig. 25. I–IV. Stages in the fusion of the steles of branch and chief axis (× 10).

I. The left-hand figure represents a transverse section of the stele of the branch with one dorsal and two marginal protoxylems; the right-hand figure represents a transverse section of the stele of the chief axis with an isolated dorsal cord. In II. fusion has taken place between the adjacent marginal protoxylems, with which also the dorsal cord of the branch stele has united. In III. the new dorsal cord is seen separating from the main xylem-mass preparatory to fusing with the already isolated dorsal cord, IV.

Figs. 26–29. *S. Braunii*, Bak.

Fig. 26. Scheme of arrangement of protoxylems (× 10).

Fig. 27. Longitudinal section of the inner cortex and pericycle showing the reticulate trabecular tissue (× 350).

Fig. 28. Transverse section of the creeping axis showing mode of origin of the vascular system of the erect shoot and root (× 20 semi-diagram).

Fig. 29. The same in longitudinal section (× 20 semi-diagram).

Fig. 30. *S. oregana*, Eat.

Transverse section of the stem. *a*, sclerotic tissue; *b*, parenchymatous cortex; *c*, *c*, leaf-traces; *d*, stele (× 60).

Figs. 31–38. *S. spinosa*, P.B.

Fig. 31. Transverse section of the stem at the level *a*–*a* in Fig. 32. The stele shows a one-layered pericycle, seven isolated patches of sieve-tubes alternate with seven protoxylem-groups and separated from the xylem by one to two layers of phloëm-parenchyma (× 350).

Fig. 32. Scheme of the arrangement of the protoxylems. The letterings *a*–*a*, *b*–*b*, *c*–*c*, *d*–*d*, *e*–*e*, *f*–*f*, correspond to the diagrammatic transverse sections in Figs. 33–38.

PLATE X.

Fig. 39. *S. spinosa*, P.B.

Transverse section of the trailing stem. *a*, pericycle; *b*, protophloëm-like elements; *c*, phloëm-parenchyma; *d*, metaxylem surrounding *e*, protoxylem (× 550).

Figs. 40–42. *S. Galeottei*, Spr.

Fig. 40. Course of the steles through an 'articulation,' and mode of fusion of the steles of axis and branch (× 10).

Fig. 41. Semi-diagrammatic transverse section of the stem between the origin of branches. *a*, hypodermis; *b*, general cortex; *c*, lacuna; *d*, pericycle; *e*, phloëm; *f*, protoxylem; *g*, metaxylem (× 50).

Fig. 42. A trabecula, showing endodermal cell and cluster of chlorophyll-bearing parenchyma abutting on the thick-walled cortex (× 550).

Fig. 43. *S. delicatissima*, A. Br.

Variations in the mode of fusion of steles of axis and branch (× 10).

Fig. 44. *S. sulcata*, Spr.
Trabecular tissue and inner cortex (× 350).

Figs. 45–48, 50. *S. Kraussiana*, A. Br.

Fig. 45. Plan of arrangement of steles. *A* = axis, *B* = branch.

Fig. 46. Inner cortex, trabeculae and stele (× 550).

Figs. 47–48. Endodermal cells enclosed by common cuticular rings (× 550). In Fig. 47 the cuticle is continued up the endodermal cells from the pericycle.

Fig. 50. Trabecular tissue, wholly or partially cuticularized, at the angles between the forking of steles (× 550).

Fig. 49. *S. rubella*, Moore.
Silicified intercellular space in cortex (× 350).

Figs. 51–55. *S. inaequalifolia*, Spr.

Fig. 51. Transverse section of the erect stem (× 50). *a*, epidermis; *b*, hypodermis; *c*, cortex; *d*, silicified layers of the inner cortex; *e*, lacuna between the ventral and ventral-accessory steles, containing completely cuticularized trabecular cells; *f*, pericycle; *g*, phloëm; *h*, phloëm-parenchyma; *i*, protoxylem; *j*, metaxylem.

Fig. 52. The dorsal and dorsal-accessory steles of Fig. 51 in transverse section (× 550). *a*, inner cortex; *b*, silicified cortical cells; *c*, pericycle; *d*, protophloëm; *e*, sieve-tubes; *f*, phloëm parenchyma; *g*, protoxylem; *h*, completely cuticularized cells in the lacuna between the steles.

Fig. 53. Longitudinal section (semi-diag.) of the stem showing the distribution of the steles at a forking of the stem. The section ends abruptly in the angle between the branch and the axis. *a*, dorsal, *b*, dorsal accessory, *c*, median, *d*, ventral, *e*, ventral accessory steles; *f*, leaf-trace from axillary leaf; *g*, stele to the dorsal, and *h*, to the ventral rhizophore (× 20).

Fig. 54. Plan of the arrangement of the steles in an erect shoot, seen from above. The steles have been turned somewhat out of their normal position to right and left in order to make it more easy to follow their course. The dotted lines indicate the borders of the ventral stele.

Fig. 55. Lacuna and trabeculae in longitudinal section (× 350).

Figs. 56–58. *S. Wallichii*, Spr.
Trabeculae and lacunar parenchyma (× 550).

PLATE XI.

Figs. 59–60. *S. Wallichii*, Spr.

Fig. 59. Transverse section of half the ventral stele (× 550). *a*, pericycle; *b*, protophloëm; *c*, sieve-tubes; *d*, phloëm-parenchyma; *e*, metaxylem; *f*, protoxylem.

Fig. 60. Apex of a primary erect shoot dissected to show arrangement of leaf-traces and protoxylems.

Figs. 61–62. *S. Willdenowii*, Bak.

Fig. 61. The serial sections I–VII illustrate the mode of fusion of the steles at the origin of a branch. The sections are taken from above, downwards.

Fig. 62. Fragmentation of the steles in a section of an old stem (× 30).

Fig. 63. *S. canaliculata*, Bak.

Lacunar tissue and endodermal cells (× 350).

Figs. 64–65. *S. Mettenii*, A. Br.

Fig. 64. Mode of fusion of the steles at the origin of a branch.

Fig. 65. Transverse section of the dorsal stele. Letters as in Fig. 59.

Fig. 66. *S. Lobbii*, Moore.

I–IV illustrate the gradual fusion of the steles in the procumbent axis from the erect axis backwards. IV *a* shows the partially sunk protoxylem at the corners of such a stele as that represented in IV.

Figs. 67–70. *S. inaequalifolia*, Spr.

Fig. 67. I–V. Serial sections illustrating the transition from the monostelic to the tristelic condition in the creeping axis.

Fig. 68. Transverse section of a small portion of the stele from the monostelic axis (× 800). *a*, metaxylem; *b*, protoxylem; *c*, phloëm parenchyma; *d*, sieve-tubes; *e*, pericycle; *f*, cuticle on the outer layer of the pericycle; *g*, inner cortex; *h*, silica.

Figs. 69–70. Cuticularized cells joining the primary and accessory steles.

Figs. 71–72. *S. viridangula*, Spr.

Fig. 71. Scheme of the mode of union of the steles at the branchings.

Fig. 72. Fragmentation of the ventral stele (× 350).

Fig. 73. *S. chilensis*, Spr.

Scheme of the mode of union of the leaf-traces and arrangement of protoxylems.

Fig. 74. *S. Lobbii*, Moore.

Transverse section of part of the stele (× 550).

Fig. 75. *S. canaliculata*, Bak.

Transverse section of the margin of the stele (550).

Fig 76. *S. laevigata*, Bak. var. *Lyallii*, Spr.

Transverse section of the stele and inner cortex of the rhizome. *a*, outer cortex; *b*, inner cortex; *c*, cuticularized endodermal cells; *d*, pericycle; *e*, sieve-tubes; *f*, phloëm-parenchyma; *g*, metaxylem; *h*, protoxylem; *i*, phloëm-parenchyma; *k*, sieve-tubes; *l*, pericycle; *m*, cuticularized endodermal cells; *n*, pericycle; *o*, sieve-tubes; *p*, phloëm-parenchyma; *r*, metaxylem (× 350).

PLATE XII.

Figs. 77–94. *S. laevigata*, Bak. var. *Lyallii*, Spr.

Fig. 77. Side view of the rhizome, erect shoots and roots (nat. size).

Fig. 78. Scheme of the arrangement of steles in a terminal branch. Each of the small circles encloses one distinct stele.

Fig. 79. End view of rhizome, &c. (nat. size).

Fig. 80. Fibres of outer cortex in transverse section showing middle lamella (× 550).

Fig. 81. Transverse section of one of the primary steles.

Fig. 82. Steles of the rhizome and of the erect axis dissected out.

Figs. 83–89. Successive sections illustrating the fusion of steles at the base of erect shoot. The primary steles are indicated by the numbers 1–4.

Figs. 90–92. Sections of the rhizome showing mode of origin of lateral shoot.

Fig. 93. Transverse section of the stele of the rhizome. (Letters *b–m*, as i Fig. 76, Pl. XI). *n*, central parenchyma; *o*, ridge of metaxylem afterwards isolated.

Fig. 94. Cuticle, epidermis, and outer cortex in transverse section (× 350).

Figs. 95 & 112. *S. Wallichii*, Spr.

Fig. 95. Surface view of epidermis (× 550).

Fig. 112. Cortical cells in long. sect. (× 350).

Fig. 96. *S. caulescens*, Spr.
Surface view of epidermis (× 550).

Figs. 97–105, 113–114. *S. Braunii*, Bak. (all × 550).

Fig. 97. Transverse section of protophloëm-elements.

Figs. 98, 99. Sieve-tubes in longitudinal section.

Fig. 100. Sieve-tubes in transverse section.

Figs. 101–105, 113. Tracheides.

Fig. 114. Protophloëm-elements.

Figs. 106, 107. *S. lepidophylla*, Spr.
Cortical cells in transverse section (× 550).

Figs. 108–110. *S. sulcata*, Spr.

Figs. 108–109. Tracheides (× 550).

Fig. 110. L. S. stele. *a*, tracheides; *b*, phloëm-parenchyma; *c*, sieve-tubes; *d*, pericycle (× 550).

Fig. 111. *S. oregana*, Eat.
Tracheae (× 550).

On Rachiopteris Williamsoni sp. nov., a New Fern from the Coal-Measures.

BY

A. C. SEWARD, M.A., F.G.S.,

University Lecturer in Botany, Cambridge.

With Plate XIII.

IN 1876[1] Professor Williamson published an account of the extremely interesting genus *Myeloxylon*, Brongniart (*Stenzelia*, Goeppert, *Myelopteris*, Renault), a plant chiefly known from fragments of petioles which have been obtained from the English Coal-measures, the famous silicified deposits of Autun, and other places. The present paper deals with one of Williamson's specimens, of which Figs. 7 and 8, Pl. II, and Fig. 17, Pl. IV, of the Memoir referred to, represent transverse and longitudinal sections.

Having obtained access to the specimens of Coal-measure plants in the collection[2] of the late Mr. Binney, I found some unusually well-preserved sections of *Myeloxylon*, and published a brief description of them in the ANNALS OF BOTANY for 1893[3]. In the same paper there were included some notes of various other examples of the same genus, including a few sections which Professor Williamson had generously placed at my disposal for examination. Among the latter I found that in some sections there were certain characters which led me to separate them from *Myeloxylon*, and to place them in the convenient genus *Rachiopteris*, as a new species, *R. Wil-*

[1] Phil. Trans., Vol. clxvi. 1876, pt. i. p. 1.

[2] In the Woodwardian Museum, Cambridge.

[3] On the genus *Myeloxylon* (Brong.): Ann. Bot., Vol. vii 1893, p. 1.

[Annals of Botany, Vol. VIII. No. XXX. June, 1894.]

liamsoni[1]. These slides were prepared from the same specimen from which Williamson's Figs. 7, 8, and 17 were drawn, and which he referred to the genus *Myelopteris* (*Stenzelia*, Goeppert, *Myeloxylon*, Brong). On suggesting to Professor Williamson that the sections differed in certain important points from the typical *Myeloxylon* structure, he re-examined them and agreed that they should be referred to another plant.

In *Myeloxylon*, it will be remembered, we have fragments of petioles showing a number of scattered vascular bundles embedded in a parenchymatous fundamental tissue in which are distributed numerous secretory canals; towards the periphery there is a characteristic form of hypodermal tissue, consisting of alternating bands of sclerenchymatous fibres and thin-walled parenchymatous cells. The vascular bundles are of the collateral type, and resemble those of cycadean petioles in the fact that the protoxylem is on the side of the xylem facing the phloëm. In discussing the affinities of the genus, the conclusion was arrived at that *Myeloxylon* 'probably occupies a position between Cycads and Ferns, but nearer to the former than to the latter.' Professor Stenzel, in referring to the affinities of *Myeloxylon* and the additional evidence afforded by my notes on the English specimens, expresses his conviction that the genus should be placed with the Cycadeae rather than in a position intermediate between Ferns and Cycads[2].

The specimens for which the name *Rachiopteris Williamsoni* has been suggested agree with the *Myeloxylon* petioles in the possession of the characteristic hypodermal tissue, and also in the general disposition of the vascular bundles in the fundamental parenchyma. On the other hand, the bundles are concentric and not collateral in form, and in addition to the reticulate, scalariform, and spiral tracheids, there is a considerable development of xylem-parenchyma. Another distinctive feature of *Rachiopteris Williamsoni* is the presence of a number of canals, arranged at fairly regular intervals in the

[1] Proc. Camb. Phil. Soc., Vol. viii. pt. ii. 1894, p. 41.

[2] Letter from Prof. Stenzel, May, 1893.

peripheral part of the phloëm of each vascular bundle; these small canals are quite distinct from the larger ones in the ground-tissue of the petiole, and do not exist in the genus *Myeloxylon*.

Description of Specimens from Professor Williamson's Collection.

In transverse section the petiole *Rachiopteris Williamsoni* presents an irregular outline, with its longest diameter about 2 cm.; the characteristic *Myeloxylon* form of hypoderm is readily detected when the sections are examined with a pocket lens. In some of the transverse sections a band of parenchymatous tissue is seen external to the hypoderm, and the outermost layer of this tissue forms a fairly well marked epidermis (Fig. 1). Internal to the hypoderm is the fundamental parenchyma with its numerous secretory canals and scattered vascular bundles, the whole arrangement being very similar to that in *Myeloxylon*. In one section (Cabinet Number 282[1]) there is seen to be a mass of parenchymatous tissue in close contact at one point with the outermost cells of the petiole. This parenchyma consists of narrow and radially elongated cells, and towards its outer edge presents a ragged appearance; associated with it is a considerable quantity of some black substance, the whole being suggestive of a scaly or laminar outgrowth from the surface of the petiole, and in part consisting of secretory cells. It is, however, impossible to give any satisfactory account of this peculiar structure, and we need not further concern ourselves with its probable nature. Fig. 2 is from a photograph of a transverse section of the petiole; the hypoderm is shown as a dark band—*h*, and the lighter coloured patch of tissue, *a*, is the parenchymatous outgrowth to which reference has just been made.

We may pass on at once to a more detailed description of the transverse and longitudinal sections.

In some parts of the periphery of one of the transverse sections (C. N. 278), there is a fairly distinct epidermal layer

[1] Prof. Williamson's Cabinet.

composed of cells smaller in size and darker in colour than the subepidermal parenchymatous elements. Fig. 1 represents a small piece of this epidermal layer with the underlying larger and more oval parenchymatous cells. The hypodermal tissue is identical with that of *Myeloxylon*, and agrees most closely with the arrangement of sclerenchyma and parenchyma, typical of *M. radiatum* (Ren.)[1]. Secretory canals are present in large numbers in this region of the petiole[2], and, as frequently happens in such structures, they contain a central core of carbonized substance, which may probably be regarded as the fossilized remains of the products of secretion; the black contents are often surrounded by a thin membrane, as previously described in the canals of *Myeloxylon*[3].

The fundamental parenchyma consists of fairly large thin-walled polygonal cells. Scattered throughout this parenchyma are a number of canals identical with those in the hypodermal tissue; in some cases they are accompanied on one side by a group of stereome-elements, as in Fig. 3; in none of them do there appear to be any particularly well-marked tangentially elongated epithelial cells, such as are usually found in *Myeloxylon*. One of these secretory receptacles, with its protective strand and fibres, has been figured by Williamson in Fig. 13, Pl. III of the *Myelopteris* memoir[4]; similar canals occur in some forms of *Myeloxylon*, as figured by Renault[5].

We must turn to the vascular bundles for the most important distinctive features of the new species. The general arrangement is much the same as in *Myeloxylon*, and some of the peripheral and smaller bundles appear at first sight to conform to the collateral type characteristic of that genus. In some cases there is the same kind of space next to the xylem, which in *Myeloxylon* marks the position of disorganized phloëm-elements; a bundle of this type is shown in Fig. 4. The large space next to the protoxylem, and towards the

[1] Etude du genre *Myelopteris*. Mém. Acad. Sci. Paris, Vol. xxii. No. 10. 1876, Pl. I.

[2] Cf. Williamson's Fig. 17, Pl. IV, *loc. cit.*

[3] Ann. Bot., Vol. vii. Pl. II, Figs. 10 & 12 *c*.

[4] Phil. Trans. *loc. cit.* [5] Plates I, III, and IV. Renault, *loc. cit.*

surface of the petiole, is probably, to a large extent, the result of shrinkage and separation of the tissues; the dark line bounding the larger tracheids shows the position of a few crushed thin-walled cells. In Fig. 5 another of the smaller bundles is shown, but here there is no vacant space; by far the greater part of the bundle consists of tracheids, and on the side facing the surface of the petiole a group of narrower elements marks the position of the protoxylem. Intermixed with the tracheids are small parenchymatous cells; surrounding the xylem is a narrow layer of thin-walled and crushed elements.

On carefully examining the tissue which, in the figure, seems to be in immediate contact with the xylem, there may be noticed at *c*, *c* two oval spaces; similar spaces occur round the entire circumference of the bundle; these are the small and characteristic canals already alluded to as one of the chief features of *Rachiopteris Williamsoni*.

In Fig. 6 a third example of the smaller bundles is represented; there is the same kind of space here as in Fig. 4, and the small protoxylem-elements are clearly defined. The chief point of interest of this figure lies in the small canals, apparently in different stages of development, shown at c^{vii}, c^{viii}, c^{ix}; these will be examined more closely after describing the larger form of vascular bundle.

Fig. 7 shows one of the larger and more central vascular bundles; the xylem-tracheids and xylem-parenchyma are clearly defined; completely surrounding these we have a band of thin-walled tissue largely consisting of elongated narrow elements, and evidently constituting the phloëm; at the outer edge of this tissue there are the characteristic canals placed at regular intervals, and at c^{iii}–c^{vi} they seem to be in similar early stages of development to those of Fig. 6. The position of the protoxylem-group is shown at p, and at p^1; the small size of the elements opposite the larger space suggests a second group of protoxylem. The two spaces are in all probability the result of tissue separation. In this, as in all the bundles of the petiole, there are no indications of a definite endodermal layer. The largest and most perfectly preserved

bundle is shown in Fig. 8; here there is an elliptical group of tracheids and xylem-parenchyma, with distinct protoxylem-elements at *p*; at *s* is a small space such as frequently occurs in the immediate neighbourhood of protoxylems, and which doubtless owes its origin to tearing. The surrounding phloëm-tissue is exceptionally well preserved, and the regularly arranged canals are very distinct. In the fundamental tissue four larger canals are shown in the figure, and on the bundle-side of each is a larger or smaller stereome-strand.

Fig. 9 affords a good illustration of the structure of a vascular bundle as seen in longitudinal section. In the centre are wide scalariform and reticulate tracheids, with rows of long and narrow xylem-parenchyma ('amylom') cells at *a*. At *ph* are the narrow cambiform elements of the phloëm, with one of the small canals at *c*. None of the longitudinal sections of these phloëm-canals reveal the existence of transverse or oblique walls; in some places, as at *b*, there are obliquely-running lines in the canal-cavity, but on closer examination these resolve themselves into cracks in the crystalline material filling up the canals. The walls of the phloëm-elements are usually somewhat crushed, but the preservation is, on the whole, remarkably good; most of the phloëm-elements are of the cambiform type, and there are no indications of sieve-tubes. The canals appear to belong to the peripheral part of the phloëm rather than to the fundamental tissue. At *f* are the cells of the latter tissue arranged in vertical rows, at *g* one large canal with the usual black contents, and at *m* the elongated elements of the stereome-strand. On the opposite side of the bundle at *n* there is a longitudinal section of what may probably be considered a sac-like receptacle, comparable to the mucilage-sacs of *Angiopteris* and other Ferns.

The section in Fig. 10 shows spiral protoxylem-elements at *p*, and at *t* a larger scalariform tracheid, with rows of xylem-parenchyma at *a*; the wall *w* does not belong to the underlying spiral element, but to some of the parenchymatous cells resting on its surface.

In addition to the spiral and scalariform elements shown in Figs. 9 and 10, the bundles contain large tracheids, with a distinctly reticulate form of pitting, like that figured in some of the *Myeloxylon* sections [1].

One of the longitudinal sections (C. N. 284) affords an example of the gradual convergence of two vascular bundles, separated by a wedge-shaped group of fundamental tissue which tapers towards the point of junction of the two concentric bundles.

To return to the bundle-canals which have been noticed in the descriptions of Figs. 5, 6, and 7. These peripheral structures are well shown in Fig. 8, *c*. Fig. 11 shows under a higher magnification the two canals c^{iii} c^{iv} of Fig. 7: at c^{iii} we have a group of small cells regularly placed round a central point; they appear to be gradually separating from the centre, and remind us of a schizogenously formed canal in an early stage of development; at c^{iv} the canal has almost reached completion. In Fig. 12 we have two more canals, c^{v}, c^{vi} of Fig. 7; c^{v} suggests a stage of development somewhat later than that of c^{iii}, Fig. 11, and at c^{vi} there are distinct indications of disorganization as well as cell-separation. Turning to Fig. 13 we find three stages of development of the canals, c^{vii}, c^{viii}, c^{ix}, shown under a lower power in Fig. 6; c^{vii} corresponds fairly closely to c^{iii} of Fig. 11 and Fig. 7; in both there is a slight accumulation of a dark substance in the space resulting from the gradual separation of the cells: c^{viii} is probably a somewhat earlier stage than c^{v} of Figs. 12 and 7, and in c^{ix} we have the same indication of disorganization as in c^{iv}, Figs. 11 and 7. It seems reasonable to conclude from the examination of a series of such examples as these, that the characteristic receptacles which accompany the vascular bundles of *Rachiopteris Williamsoni* are of the nature of small secretory canals formed as the result of a schizolysigenous process [2]. In one slide (C. N. 284) there is a solitary instance of a bundle-canal containing dark-coloured contents

[1] E. g. Ann. Bot., Vol. vii. Pl. II, Fig. 18.

[2] Cf. Tschirch, Angewandte Pflanzenanatomie, 1889, p. 517.

similar to that in the larger canals of the fundamental tissue, and possibly representing the remnants of secretion.

It remains for us to consider the probable affinities of the plant whose histological structure has been described in some detail. Its resemblances to *Myeloxylon* have already been referred to; how far these should be looked upon as a proof of close relationship it is difficult to say. Possibly the acquisition of fresh material may help to bridge over such differences as are at present apparent between the two fossils; in any case it seems advisable to give expression to the well-marked characteristics of the present species by adopting a distinctive name.

In *Myeloxylon* the collateral bundles and the position of the protoxylem are two important features; these, as well as the nature of the canals, are strongly in favour of cycadean affinities. In *Rachiopteris Williamsoni* the bundles are concentric, there is a considerable development of xylem-parenchyma, the canals of the fundamental tissue do not appear to have the same distinct epithelial layer which is so clearly marked in *Myeloxylon* and recent Cycads; and, finally, there are the smaller canals in the peripheral part of the phloëm of each bundle. How far are these differences of taxonomic value?

The fact of a vascular bundle being of the collateral or concentric form is probably of no very great importance; in recent Ferns we have the exceedingly common occurrence of collateral bundles in the finer branches of the fronds which have concentric bundles in their larger axes. In *Rachiopteris Williamsoni* some of the peripheral bundles approach much more closely to the collateral type than the larger and more distinctly concentric bundles. Haberlandt notes the occurrence of collateral bundles in the fronds of *Marattia laxa* and *Angiopteris longifolia* among the Marattiaceae[1], and Mr. Brebner, who is at present engaged in a detailed examination of Marattiaceous petioles, tells me he finds similar bundles in *Marattia fraxinea*. Granting this frequent

[1] Sitzgber. d. k. Akad. Wiss. Vienna, Abth. i. 1881, p. 7.

association of concentric and collateral vascular strands in fern-fronds, there is a considerable difference between the distinctly collateral bundles of *Myeloxylon* and the more typical fern-like bundles of *Rachiopteris*. As regards the presence or absence of xylem-parenchyma, it is a fact on which very great stress must not be laid. In the petioles of Marattiaceae we occasionally find a few parenchymatous elements associated with the xylem-tracheids, but as a rule they are absent. De Bary[1], in speaking of concentric bundles, refers to those in which the xylem is entirely composed of tracheids, and those in which rows of parenchymatous cells are intermixed with them: he says—'The two conditions are distributed according to species and perhaps genera, not according to the form of the bundle.' Potonié[2], in a useful paper *Ueber die Zusammensetzung der Leitbündel bei den Gefässkryptogamen*, makes use of Troschel's term 'amylom' for xylem-parenchyma, and, among other examples, he cites the occurrence of such tissue in the bundles of *Marattia laxa*[3]; he shows that amylom-cells may be present or absent in different parts of the vascular system of the same plant. Troschel's term is applied not merely to those parenchymatous cells which are intermixed with the tracheids, but includes those immediately surrounding the tracheid-group; in speaking of the occurrence of such elements in the fossil petioles and the recent Marattiaceae it is only the amylom-cells intermixed with the tracheids that are referred to. The marked contrast between *Myeloxylon* and *Rachiopteris Williamsoni* as regards the xylem-parenchyma should probably be kept in view as a distinction of some importance in questions of affinity. On comparing the sections of *R. Williamsoni* with those of recent fern-petioles, we find a much closer correspondence with such a Fern as *Angiopteris* than with any of the Leptosporangiate genera. It is true that there may be a natural bias in favour of the

[1] Comp. Anat., p. 344.
[2] Jahrb. k. bot. Gart. und bot. Mus. Berlin, II, 1883.
[3] Potonié, *loc. cit.* p. 244: Pl. VIII, Fig. 4.

Marattiaceae when we are dealing with Palaeozoic Ferns, but in spite of this there seems little doubt that we must regard this new Carboniferous type as more closely connected with Marattiaceae on histological grounds than with any other family. In the monograph on the Marattiaceae by De Vriese and Harting there is a figure of a transverse section of an oval vascular bundle of *Angiopteris Teysmanniana*[1] (= *Angiopteris evecta*[2], var. *Teysmanniana*), which shows a series of 'canals' disposed at regular intervals in the phloëm; these are described as small canals containing a yellow juice holding small globules in suspension. The phloëm is spoken of as a 'gaine cellulaire.' On examining sections of *Angiopteris evecta* var. *Teysmanniana*, for which I am indebted to the Director of the Royal Gardens, Kew, I find a regular arrangement of such elements as are represented by De Vriese and Harting; in longitudinal section they have the form of fairly wide tubes with oblique cross-walls, and contain a considerable quantity of small yellowish globules. They are probably sieve-tubes of a type unusual in Ferns, and not canals as described by De Vriese and Harting. Fig. 14 shows the large and conspicuous form of these peculiar elements; opposite the protoxylem there is an indication of cell-separation ('Lückenparenchym' of Russow) such as frequently occurs in the fossil bundles.

The bundle-canals of *Rachiopteris Williamsoni* cannot be regarded as homologous in structure with the large sieve-tubes of *Angiopteris*; their mature form and their manner of development are strongly suggestive of small secretory canals. In the vascular bundles of petioles of *Osmunda regalis*, we find groups of tannin-sacs on the concave side of the curved bundle[3], and to some extent on the convex side; these may

[1] Monographie des Marattiacées, 1853, p. 24, Pl. VII, Fig. 17.

[2] In the Synopsis Filicum, 1868, p. 440, all the species of De Vriese and Harting are included under *A. evecta*, Hoffm.

[3] Thomae, Pringsh. Jahrb. Vol. xvii. 1886, Pl. VII, Fig. 3 a. Kühn also refers to these sacs in *Todea barbara*. Untersuchungen über die Anatomie der Marattiaceen und anderer Gefässkryptogamen, p. 35: Inaug. Dissert. Marburg, 1889. (Flora, 1889. p. 457.)

be compared with the bundle-canals in the fossil petiole. In his description of the gum-canals of *Angiopteris evecta*, Frank[1] speaks of two kinds, and gives an account of their development. Kühn arrives at different results from those of Frank as regards the canal-development, and calls attention to the absence of epithelial cells[2]. This absence of a distinct epithelial layer lining the canal cavity in Marattiaceae agrees with the canal-structure in *Rachiopteris Williamsoni*.

The existence of a stereome-group of elements in association with some of the larger canals in the fossil species is a character which we meet with in *Angiopteris*, *Marattia* and *Danaea*[3].

There is the further question as to the apparent absence of an endodermal layer in *R. Williamsoni*; in recent Marattiaceae the vascular bundles of the petioles are often described as having no distinct endodermis. Van Tieghem[4] speaks of the steles of Marattiaceous petioles as having no well-marked 'plissements' on the walls of the endodermis, except in the case of *Danaea*. Leclerc du Sablon[5] points out that an endodermis exists in *Angiopteris evecta*, but its cells are only recognized after treatment with certain reagents. We may consider, therefore, that for purposes of comparison with fossil petioles, there is no endodermis in Marattiaceae, except, perhaps, in the case of *Danaea*. The preservation of the fossil vascular bundles is in this case so exceedingly good, that, if a typical endodermis were present, it would not be too much to expect that some indications of the usual characters might be detected in the mineralized tissue, especially in view of the fact that Hovelacque and Bower have recognized endodermal cells in Lepidodendroid axes.

On the whole, then, I regard *Rachiopteris Williamsoni* as a fern-petiole, which agrees in the histological details of structure more closely with the petioles of Marattiaceae than

[1] Beiträge zur Pflanzenphysiologie, Bd. ii. 1868, p. 112.
[2] Kühn, *loc. cit.* pp. 32, 51.
[3] My thanks are due to Prof. Bower for specimens of this genus.
[4] Trait. Bot. Vol. ii 1891, p. 1388.
[5] Ann. Sci. Nat., Bot., Sér. II. Vol. xi. 1890, p. 12, Pl. II, Figs. 29–31.

with any other living plants. It would be unwise to definitely include it in that family; the evidence at our command is far too incomplete, and it is probable that those peculiarities of structure which have been described in the fossil petiole are of sufficient importance to exclude it from any existing family.

I have ventured to adopt the specific name *Williamsoni* as a slight recognition of the encouragement and generous help which I have always received from Prof. Williamson.

EXPLANATION OF FIGURES IN PLATE XIII.

Illustrating Mr. Seward's paper on *Rachiopteris Williamsoni*.

The numbers in brackets refer to Prof. Williamson's Catalogue of his private collection.

Fig. 1. Transverse section of the outermost cells of a petiole, showing the epidermal layer *ep*. ×175. (C. N. 278.)

Fig. 2. Transverse section of a petiole of *R. Williamsoni*: *h*, hypoderm, nat. size; *a*, parenchymatous tissue and secretion? *sh*, section of a shell. (C. N. 282.)

Fig. 3. Canal with stereome. ×175. (C. N. 282.)

Fig. 4. Single vascular bundle near the periphery of the petiole. ×37. (C. N. 282.)

(For this photograph, and that reproduced in Fig. 5, I am indebted to Mr. C. A. Barber.)

Fig. 5. Single bundle: *p*, protoxylem; *c*, *c*, bundle-canals. ×37. (C. N. 282.)

Fig. 6. Single bundle: *c*, c^{I} and c^{II}, canals. ×37. (C. N. 282.)

Fig. 7. A larger vascular bundle: c^{III}–c^{VI}, canals; *p*, protoxylem; p^{I}, ? protoxylem. ×37. (C. N. 282.)

Fig. 8. Single large bundle: *s*, space close to protoxylem; *c* . . ., bundle-canals. ×37. (C. N. 282.)

Fig. 9. Longitudinal section of a vascular bundle: *a*, xylem-parenchyma; *ph*, cambiform phloëm-elements; *c*, bundle-canal; *b*, crack in crystalline substance filling up canal; *f*, fundamental parenchyma; *g*, large canal; *m*, stereome-strand; *n*, sac. ×37. (C. N. 284.)

Fig. 10. Longitudinal section of the protoxylem of a bundle: *p*, protoxylem; *t*, scalariform tracheid. ×175. (C. N. 285.)

Fig. 11. Two canals, c^{III} and c^{IV} of Fig. 7. ×175.

Fig. 12. Two more canals, c^{V}, c^{VI} of Fig. 7. ×175.

Fig. 13. Three canals, c^{VII}, c^{VIII}, c^{IX}, of Fig 6. ×175.

Fig. 14. Transverse section of *Angiopteris evecta*, var. *Teysmanniana*, showing a single vascular bundle with large sieve-tubes in the phloëm: *sv*, sieve-tubes. ×37.

On the Occurrence of Centrospheres in Pellia epiphylla, Nees.

BY

J. BRETLAND FARMER, M.A.,

Assistant Professor of Biology, Royal College of Science, London,

AND

JESSE REEVES,

Marshall Scholar, Royal College of Science, London.

With Plate XIV.

IT is a well-known fact that the spores of *Pellia epiphylla*, like those of some other Liverworts, germinate while still enclosed in the sporogonium, and that long before the wall of the capsule ruptures, the spores have become multicellular bodies. The spores of *Pellia*, when in this condition, afford excellent material for the study of the karyokinesis, owing to the large size of their nucleus and the relatively small number of its chromosomes. But the special interest which attaches to them lies in the singular degree of clearness with which the attraction-spheres (or centrospheres) are differentiated, and in the facility which is thereby afforded for the study of these structures in their relation to the process of nuclear division.

For the purpose of this investigation, fruiting plants of *Pellia* were gathered during the winter, and were preserved

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in strong spirit, which, so far as the present plant is concerned, we find to give results in no way inferior to those obtained by the use of absolute alcohol. The sporogonia, with their contained spores, were embedded in paraffin, and cut with the microtome in the usual way. For staining purposes we employed a considerable variety of reagents. but we obtained the best results after using the following double stains;—gentian violet and orange G., gentian violet and eosin, aniline blue and acid fuchsin. In every instance we used the stains successively, and on the whole the second of the above combinations gave the most satisfactory preparations. It should be noted, however, that in dealing with these structures, we find it by no means follows that a given treatment which may answer admirably for one plant, will succeed equally well in the case of another, and in this we are in accord with the experience of those who have devoted attention to animal centrosomes. The reason of the difference is probably to be sought in the slight chemical or physical differences which exist between the protoplasmic structures in different organisms, but our knowledge of the intimate constitution of these bodies is at present so scanty that we are compelled to have recourse to empirical experiment, in order to determine which reagent may be the best to use in each particular case.

The spores of *Pellia epiphylla* are crowded with starch-grains, and contain, as has been already said, a nucleus of considerable size, in which the chromatin occurs in a condition of aggregation during the resting stage. The nuclear membrane is extremely sharply defined, and within the body of the nucleus a nucleolus may be seen, though it is not always easy to distinguish.

When nuclear division is about to take place, two structures of a minute size appear on the outside of, and in contact with, the nuclear wall, and from them beautiful radiations extend. These bodies, or centrospheres, are commonly seen to be diametrically opposite to each other in position, for we have not succeeded in demonstrating them in the perfectly resting

cells, nor have we been able to ascertain the existence of any definite particle within them which would indicate the presence of a centrosome; it is true that in some instances such a point could be distinguished, but we do not attach much importance to it, since in the great majority of centrospheres it completely eluded recognition. And, although the radiation in the cytoplasm may readily be traced into the protoplasmic corpuscle, we prefer for the present, at any rate, to term this a centrosphere rather than a centrosome, since it appears probable that the corpuscle is really equivalent to something more than the centrosome alone.

The cytoplasmic radiations are exceedingly clear in this plant, and especially well seen when regarded from above, i.e. in the direction of the polar axis, and they are then seen to spread out in a star-like manner from a common centre (Pl. XIV, Fig. 5).

Presently the centrospheres assume such a relation to the nucleus that one can hardly resist interpreting it as a pulling strain. The nucleus becomes more and more drawn out into an elliptical shape, and at the somewhat pointed ends the centrospheres are respectively situated (Pl. XIV, Fig. 2). The chromatin now becomes distributed in the form of a narrow equatorial band lying just within the nuclear membrane which still persists, and indeed, in this equatorial region, exhibits, if possible, a sharper outline than before. The chromatin gradually becomes more definitely fibrillar, and finally appears in the form of eight chromosomes, which can easily be counted when the equatorial nuclear plate is formed. Meanwhile the rest of the nucleus is entirely free from staining-substance, and its ends become more and more drawn out, whilst its wall becomes *pari passu*, much thinner, except just outside the chromatic band mentioned above. The cytoplasmic radiations increase in number and distinctness, and extend over the attenuated nuclear wall. Concomitantly with the final disappearance of the latter, the achromatic spindle is differentiated, and rapidly becomes very prominent, owing to its growing capacity for taking stains. It is hardly possible,

in a case like the present, to escape the conviction that there exists a close relation between the spindle and the nuclear wall, over which the radiations have been extending. The centrospheres frequently become difficult to distinguish at this stage, and the polar radiations are often hardly visible. The achromatic spindle apparently ends sharply at a point, but we have convinced ourselves, as the result of a careful examination of a somewhat extensive series of preparations, that the spindle does not, as a matter of fact, terminate in the general cytoplasm, but that its two apices are respectively occupied by two (sometimes only one) corpuscles, and we believe we are justified in regarding these as the representatives of the centrospheres, although they do not exhibit a degree of clearness such as that described by Schottländer[1] for the antherozoid-mother-cells of *Marchantia*. On the other hand, the radiations are obvious here, while Schottländer states that with one exception these did not occur in the plants investigated by him.

After the separation of the chromosomes from the equator to form the daughter nuclei, a beautiful cell-plate is formed across the achromatic spindle, by local thickening of the 'Verbindungsfäden,' and it then extends centrifugally, finally meeting the peripheral wall of the cell, exactly as in the higher plants.

During the reformation of the daughter-nuclei the radiations above spoken of at first become again more distinct, but they finally die away altogether, and in the completely resting nuclei they are entirely wanting. When this stage is reached we do not succeed in definitely detecting the presence of a centrosphere or centrosome, and we do not regard it as probable that these structures retain their individuality within the cytoplasm in the case of *Pellia*. It is easy enough to find bodies of minute size, surrounded by their diffraction areas, but it would be pure empiricism to fix on any one or

[1] Schottländer, Beitr. z. Kentniss d. Zellkerne u. d. Sex. Zell. b. d. Kryptogamen. Cohn, Beitr. z. Biol. d. Pflanzen, Bd. VI, 1892.

two of these and call them centrosomes. Nor is this surprising, since even in many favourable cases of cell-division in animals it is by no means clear as to what is the fate of these bodies, whether they become diffused in the general cytoplasm, or whether they become retracted into the nucleus. If they really do persist in *Pellia*, the latter view seems perhaps the least improbable, owing to the intimate relations in space which always obtain between nucleus and centrospheres in this plant, and which have been already alluded to.

The processes of division, as described above, are subject to some deviations. In a few instances, one or both of the centrospheres, with their attendant radiations, were seen in positions apparently remote from the nuclear wall, during the earliest phases of division. This appearance proved, however, on examination, to be illusory, and to be due to the fact that the centrospheres had not taken up diametrically opposite positions outside the nucleus, so that when this body begins to be drawn out, it assumes an asymmetrical, or more correctly stated, a bilaterally symmetrical shape (Figs. 3, 4). If, then, the distortion is very great, and the observer happens to regard the nucleus from the convex side, it is easy to overlook the oblique prolongations of the membrane, and hence the asters appear to be situated in the cytoplasm and away from the nucleus (Fig. 4). It is worth notice that in all asymmetrical nuclei of this nature the belt of chromatin is always broader on the outer than on the inner curve.

When the asymmetrical condition becomes very strongly marked, a third centrosphere, often feebler than the other two, may be occasionally observed. We have not seen any simultaneous division of the nucleus into three daughter nuclei, although occasionally the arrangement of the chromatin is such as to suggest that this might occur; as an alternative, it is possible that the weakest centrosphere ultimately becomes absorbed by one of the other two, and this we regard as the most likely explanation of the fact that even in preparations which exhibited three centrospheres, we never saw three *spindles*, or any such arrangement of cell-walls in completely

divided cells as would support the view of a simultaneous division having taken place.

It is impossible to avoid being struck by the differences existing between the processes accompanying nuclear division in the spores of this Liverwort, and those obtaining in certain other plants. We reserve, however, the consideration of these for a future paper, contenting ourselves in this place with a description of the facts as they may be observed in *Pellia epiphylla*.

EXPLANATION OF FIGURES IN PLATE XIV.

Illustrating Messrs. Farmer and Reeves' paper on the Occurrence of Centrospheres in *Pellia epiphylla*, Nees.

All the figures refer to *Pellia*, and are drawn from spores germinating within the sporogonium.

Fig. 1. Mature spore with the nucleus in the resting condition.

Fig. 2. Nucleus becoming elliptical. The centrospheres at the two poles. The chromatin aggregated in an equatorial belt.

Fig. 3 and 4. Nucleus assuming an asymmetrical shape. The centrospheres not diametrically opposed.

Fig. 5. A polar view of the centrosphere and the radiations.

Fig. 6 and 7. Nucleus in which chromatin fibrils are becoming clear.

Fig. 8. Later stage of karyokinesis.

Fig. 9. Chromosomes, eight in number, seen in polar view.

Fig. 10. Daughter-nuclei in process of reformation. A marked cell plate.

Fig. 11. Spore which has divided into three cells, in each of which stages of nuclear division are shown.

NOTES.

ON THE GERMINATION OF THE POLLEN-GRAIN AND THE NUTRITION OF THE POLLEN-TUBE [1].—The germination of the pollen-grain, leading to the protrusion of a pollen-tube, suggests, at once a process analogous to the formation of the prothallium of the Vascular Cryptogams. Its peculiar situation and the fact that it has to make its way, unlike the latter, through a mass of tissue, suggest however certain peculiarities attending its nutrition. The absence of chlorophyll and the probable richness of the environment in various elaborated materials makes it probable that the progress of the tube downwards through the style is attended by a process of intra- or extra-cellular digestion, depending on the occurrence and activity of enzymes.

It has been shown by various observers that pollen-grains allowed to grow in solutions of cane-sugar speedily bring about the appearance of a reducing sugar. Even when germination has been inhibited by antiseptics, the same inversion of the cane-sugar has been observed to take place. Certain grains again when cultivated in weak starch-paste have been shown to liquify it, with formation of maltose.

The research which forms the subject of the present paper was directed first to the preparation and identification of digestive enzymes from pollen, and to the variations in their amount which attended the progress of the pollen-tube through the tissue of the style.

Both diastase and invertase were found to exist in various pollens; some containing both, some only one of the two. To extract them the pollen was collected from dehiscing stamens, bruised in an agate mortar, and extracted with various solvents, usually 5 per cent. of NaCl. As an antiseptic during the digestions, ·2 per cent. KCy was found most serviceable.

[1] Abstract of a paper read before the Royal Society, February 8, 1894; see also Annals of Botany, Vol. v. 1891.

The ground pollen after a few hours' exposure to the solvent was filtered off and the filtrate tested by allowing it to remain in contact with cane-sugar in solution or starch-paste of 1 per cent. strength for some hours. The reducing sugar was estimated by titration with Fehling's solution. Details of the various experiments made are given at some length in the paper.

Diastase was by this method prepared from the pollen of *Lilium*, *Helleborus*, *Helianthus*, *Gladiolus*, *Anemone*, *Antirrhinum*, *Tropaeolum*, *Pelargonium*, *Crocus*, *Brownea*, *Alnus*, *Tulipa*, and *Clivia*. It is very widely distributed, very few pollens examined yielding no result. Invertase was found in the pollen of *Helleborus*, *Narcissus*, *Richardia*, *Lilium*, and *Zamia*.

During the germination of the pollen the quantity of both enzymes was found to be considerably increased; in some cases four or five-fold. The difficulty of extraction was greater in the case of the ungerminated grain, the thin-walled pollen-tube yielding it to the solvent fairly easily. The facility of extraction was shown, however, not to be the explanation of the greater activity of the latter extract, but a definite increase in amount was made evident, the increase being generally greater when the tubes were grown in a nutritive fluid than when they were cultivated in distilled water.

In one case the increase was found to be preceded by an initial diminution, which lasted only till the tube was about four or five times the length of the diameter of the pollen-grain.

When the power of germination of the grain was becoming feeble, which usually took place about three weeks after collection, the amount of enzyme contained in the pollen was very considerably diminished.

Further experiments were carried out to trace the changes in the contents of the grains and tubes as the germination proceeded, and to see what was taking place in the cells of the style during the same period.

The contents of the pollen-tube were generally granular, the protoplasm showing streaming movements. Besides the granularity of the latter, however, larger refringent granules were observed towards or actually at the tip of the tube, which were being continuously or intermittently extruded into the culture-fluid. In one case (in *Narcissus*) this extension was observed to take place through a pore with well-defined lips at the tip of the tube. The granules so extruded

were remarkably like those noticed by Marshall Ward, as put out of the hyphae of *Botrytis*, and were probably, as in the latter case, the enzyme itself. The extrusion of the contents of the pollen-tube by certain definite pores has already been described by Van Tieghem.

When pollen-grains were treated with a strong solution of chloral hydrate in which a little iodine had been dissolved, they were found to contain in many cases large quantities of starch in very minute grains. Other experiments, of somewhat complicated nature, showed that many contained, either with or without starch, various quantities of cane-sugar, glucose, and maltose. During germination the starch-grains were found to pass in a sort of streaming motion down the tube, and to be digested as they went. When the tubes were of some considerable length, iodine coloured the granules blue close to the pollen-grain, violet and purple further down the tube, and nearly red close to the tip, indicating the gradual hydrolysis of the starch and the coincident formation of dextrin.

The style of the Lily was the one chiefly examined for evidence of the disposition of nutritive material in this organ. It was found to have a very definite relation to the progress of the pollen-tube. The style of the Lily contains a canal continuous with the cavity of the ovary and opening outwards at the surface of the stigma. This canal shows a very delicate epithelial lining, the cells being somewhat papillate. There are three fibro-vascular bundles running up it, placed symmetrically.

The cells of the epithelium and of three or four rows of the loose conducting tissue immediately underlying it were found to be the great seat of the storage of starch. In some styles they were quite full, turning almost black when treated with iodine. The soft tissue round the bundles was also full of it, indicating a definite deposition in the style of starch originally formed elsewhere. The starch did not reach quite to the stigma, but stopped short a little below it. The conclusion apparently led to by a consideration of the disposition of the starch in the grain and in the style is that in both it serves as reserve nutritive material, the grain on germinating using up first its own reserves by intra-cellular digestion and then being fed by the starch of the style which is digested in large measure by the diastase excreted from the tip of the tube. The initial diminution of diastase, already mentioned, occurs while the intra-cellular digestion of the store in the pollen is proceeding, the subsequent increase being connected with

a continuous excretion into the tissue of the style to act upon the reserves deposited there.

In addition to this excreted diastase, in certain cases the style itself secretes the same enzyme, the quantity being greatest while the style is young and diminishing after fertilization has been effected.

Besides starch, styles of various plants were found to contain cane-sugar, maltose, and possibly glucose.

The nutrition of the tube is consequently a process in which both the grain itself and the tissue through which it grows take a part; both contain reserve materials and enzymes, though the latter are much more abundant in the pollen than in the styles.

The absorption of nutritive material by the tube in most cultures is followed by an increase in the amount of reserve material deposited there as starch. In some cases (as *Zamia*) the resting grain contains neither starch nor diastase. On its absorbing sugar-solution, however, starch makes its appearance, and later, diastase can be detected.

The formation of the enzymes is therefore largely helped by the absorption of nutriment, which seems to stimulate the pollen-grain to produce them.

This investigation was carried on in the Jodrell Laboratory of the Royal Gardens, Kew.

J. REYNOLDS GREEN, London.

BOTANICAL NOTES, No. 6: ON THE EXTRA-FLORAL NECTARIES OF ALEURITES.—It seemed possible that an examination of the relations which laticiferous tubes bear to the nectaries of a plant, might throw some light on the vexed question as to whether the tubes conduct carbohydrates or not[1]. I selected for observation a plant of *Aleurites cordata* (Euphorbiaceae) which was growing in the gardens at Whampoa.

Aleurites cordata has large, long-stalked, palmati-lobed leaves. The large veins terminate in the angles between the lobes, and at the end of each vein stands a sessile nectary. In addition, erect, stalked nectaries are situated on the petiole at its point of junction with the lamina.

Some of the leaves of this plant are not lobed, but are entire and

[1] See papers by Dr. Scott, and by myself (with literature). Annals of Botany, Vol. iii. 1889.

broadly ovate. And on such leaves only the two petiolar nectaries are found[1]. The present observations relate to the petiolar nectaries of *Aleurites*.

Each nectary is a green, stalked, shallow basin, the concavity of which is tinted red. The secreting cells which line the basin, form a single layer of palisade-like cells. The general cuticle is preserved over these, and the secretion emerges through splits in it. The main body of the basin is composed of an anastomosing system of conducting parenchyma and ground-parenchyma. The cells of the latter are larger and stain less deeply than those of the former. The conducting parenchyma is roughly arranged in a fan-like manner beneath the secreting layer. But at least one layer of cells differing from both types of parenchyma mentioned, is interposed below the secreting layer. Its cells are united to form a layer in which there are no intercellular spaces. Lower down towards the stalk of the nectary, the conducting parenchyma-cells surround successively tracheides and spiral vessels, and then complete bundles. In the stalk itself the vascular bundles roughly form a ring which divides the ground-tissue into a 'cortex' and a 'pith.'

In the petiole there are numerous very large laticiferous tubes which send off a few (about five or six) narrow branches into the nectary. These finer tubes give off few or no branches in the nectary, and some of them end bluntly under the secreting layer. Owing to their poverty in numbers and unbranched condition, the anatomical connexion between the tubes and the secreting cells is very incomplete. The secreting cells contain proteids, sugar, a red colouring-matter (a compound of tannin?), tannin, but no starch. In the ground-parenchyma starch, tannin, and crystals of calcic oxalate occur. The conducting parenchyma contains sugar, but no starch or crystals. In the laticiferous tubes tannin is found, but no starch.

Darkening the nectaries of leaves on the plant or of excised leaves, or darkening the whole leaves, caused a gradual disappearance of the

[1] This interesting correlation of the lobing with the occurrence of marginal glands in the angles is also well illustrated by three species of *Croton* growing wild in Hong Kong. In *Croton lachnocarpum* (Benth.) the leaves are serrulate-crenate, and at the serratures are small stalked glands directed downwards. In *Croton chinense* (Benth.) the leaves are serrate or entire, and the glands are fewer and smaller. Finally, in *Croton Hancei* (Benth.) the leaves are entire or minutely serrate, and each leaf has occasionally two minute glands at the base of its lamina, sometimes even these are absent.

starch, but the nectaries continued to excrete for a time. There was not the slightest indication of any connexion between the mode of disappearance of the starch and the arrangement of the laticiferous tubes. The starch gradually disappeared from the ground-tissue of the basin, remaining longest near the margin. Thus physiological evidence confirms the conclusion suggested by the anatomical fact that but few branches of the laticiferous tubes are distributed to the nectary. Unfortunately in this plant the ground-tissue is intermingled with the conducting parenchyma in such a manner as to render the nectaries somewhat unfavourable objects for tracing the exact track of the starch in the parenchyma. But the method here suggested may possibly be applied with greater success by others who have at their command a larger choice of laticiferous plants which possess nectaries.

PERCY GROOM, Oxford.

On the Comparative Anatomy of the Casuarineae, with special reference to the Gnetaceae and Cupuliferae.

BY

L. A. BOODLE and W. C. WORSDELL.

With Plates XV and XVI.

DURING the last half-century the anatomy of *Casuarina* has been investigated by numerous authors. Some of these have restricted their accounts to the description of special points, e. g. the formation of periderm, &c., while others have given us a more general survey of the whole structure.

Goeppert[1] published a paper in 1841 on the anatomy of several species of *Casuarina*. He gives a fairly accurate description of the chief tissues of the woody stem, and was the first to point out the peculiar formation of the parenchymatous system, which is so characteristic of this plant, and on which he lays especial stress. As was perhaps not unnatural, however, having regard to the time at which the paper was written, he missed the significance of certain peculiarities of the structure, which we shall have occasion to refer to in the course of this paper.

[1] Linnaea, 1841 : also Ann. Sci. Nat., Bot., 1842, 2 sér. tome XVIII.

In 1855 there appeared a short thesis by Stache[1] on living and fossil Casuarineae.

Sanio[2], in 1860, in a long paper on the formation of cork in various plants, describes minutely the development of the periderm in *Casuarina*, giving a very clear figure showing the mode in which it cuts off the ridge of assimilating tissue from the main stem. In a subsequent series of valuable papers[3], in which he describes the different elements which compose the woody tissues of plants, he gives repeated references to *Casuarina*, of which the most important are those in which the nature and morphology of the fibrous tracheides and parenchymatous tissues are touched upon.

By far the most detailed and fullest account of the anatomy of these plants is found in a long thesis by Loew[4], published in 1865. Without following the paper through all the points it touches upon, we will just mention that the author has a long chapter on the subject of the medullary rays and the concentric bands of parenchyma, and he speaks of the ridge of assimilating tissue as being beyond dispute of the nature of a leaf, while he gives a short, though exact description, which no other author has done, of those elements in the ridge, which we, in the following pages, have described as 'transfusion-tissue.'

Poisson's paper[5], which came out in 1874, is largely taken up with reviewing and summarizing the results of Loew's investigations, stating, as the most original part of that author's work, his attempt to classify the Casuarinas according to the form and structure of the ridges or 'phyllichnia'; the second part of the paper is a description of the reproductive organs, and a monograph of the genus, which he separates into two divisions: Cylindricae or Cryptostomae, and Tetragonae or Gymnostomae.

[1] De Casuar. nunc viv. et foss. nonn., Vratisl., 1855.
[2] Jahrb. für wissenschaftl. Bot., 1860, Vol. ii, p. 103.
[3] Bot. Zeitung, 1863.
[4] De Casuar. caul. fol. evolut. et struct. Berol., 1865.
[5] Recherches sur les Casuarina, Nouv. Arch. du Muséum, Vol. x, 1874.

Schube[1], in a dissertation on the anatomy of leafless plants, devotes a little space to the description of the assimilating stem of *Casuarina*.

One of the most interesting treatises on the subject of *Casuarina* is the paper by Lecomte[2] in 1886, in which is a concise account of the main features of the stem-structure. Although he refers frequently to Loew's paper with regard to other matters, when he comes to indicate, in a rather obscure way, the presence of the 'transfusion-tissue,' there is no reference whatever to that author's more complete account of this same tissue. He accounts, in an adequate manner, which Goeppert had failed to do, for the appearance of the tracheides in the large medullary ray, and finally refers to the investigations of Sanio and Poisson with regard to the formation of cork in the young stem.

A paper by H. Ross[3], on the assimilating tissue and cork-formation of various leafless plants, has a few pages devoted to *Casuarina*, which are chiefly occupied with a consideration of the periderm-structure. We have had occasion, during our investigations of *Casuarina*, to refer several times to Solereder's[4] important classification of Dicotyledonous plants according to the structure of their wood. Though it is little more than a grouping together of plants having certain characters in common, it affords an excellent table of reference; while, in its minute investigation of the structure of the separate elements which make up the wood, it has also an important and valuable bearing on the subject of our work.

Last year there appeared a paper by C. Houlbert[5], entitled Recherches sur le bois secondaire des Apétales, in which he shortly describes the anatomy of various species of *Casuarina*, and attempts to give that genus a systematic

[1] Beitr. zur Kenntn. der Anat. blattarmer Pflanzen, 1885.
[2] Bullet. de la Soc. Bot. de France, Vol. viii, 1886.
[3] Nuovo Giorn. Bot. Ital., Vol. xxi, 1889.
[4] Ueb. d. systemat. Werth d. Holzstruct. b. d. Dicot., 1885.
[5] Ann. des Sci. Nat., Bot., Vol. xvii, Nos. 1, 2, 1893.

position founded on anatomical characters. His descriptions of the tissues are, however, very general. Like others before him, he comes to the conclusion that the structure of *Casuarina* is isolated among the Apetalae, and perhaps among Dicotyledons. He places it nearest to Proteaceae.

The general character of the young stem of *Casuarina* is sufficiently well known; so that we need only shortly mention, that the young branches are the chief assimilating organs of the plant; this assimilating function they carry on by means of the characteristic ridges which run in a longitudinal direction down the stem, and have been described as adherent portions of the rudimentary scale-like leaves, which are arranged in whorls at intervals on the stem.

Anatomy of Young Stem.

The transverse section of the young stem of *Casuarina*, as seen in Plate XV, Fig. 1, shows a number of prominent ridges, varying in number and shape according to the species. They are separated from one another by furrows, which also in their form offer considerable variation. Poisson[1] describes another division of *Casuarina*,—Tetragonae,—in which depressions or furrows are absent; but in the species described by him the position where the furrows would ordinarily occur is indicated by a tissue of colourless cells separating the palisade-tissue of each corner; we find however, in another species, *C. Rumphiana*, Miq., described by Lecomte[2], the palisade-tissue forms a continuous layer round the stem.

The ridges are covered with a rather small-celled epidermis, having a thick outer wall in which small, refractive, roundish bodies are embedded: they lie in pocket-like depressions, and are arranged in fairly regular longitudinal rows on the stem. On the sides of the ridges, in the furrows, transversely-placed stomata occur; they belong each to a longitudinal row running down the stem. A tuft of hairs, each usually con-

[1] Loc. cit., p. 103. [2] Loc. cit., p. 314.

sisting of three or four cells with thickened walls, arises from the epidermal cells at the base of the furrow. If the epidermis lining the furrow were removed and laid out flat, one would see a row of hairs down the centre with three to four rows of stomata on either side. The function of the hairs is evidently to protect the stomata and to diminish transpiration. The slight hairiness of the stem of many species is due to the projection of these hairs beyond the opening of the furrow.

In the square-stemmed species described by Poisson, where no furrows occur, the stomata are placed in long longitudinal rows, at more or less frequent intervals round the stem. Thus, except for being slightly more depressed, the stomata of these species entirely miss the protection of the furrow and the hairs.

On macerating a piece of stem in Schultze's mixture, the cuticle splits at the line of insertion of the hairs, and so peels off in strips. Immediately below the epidermis, in a transverse section, there is a narrow band of sclerenchyma, which in some species (e.g. *C. glauca*, Sieb. and *C. torulosa*, Ait.) is prolonged into a conspicuous ridge, penetrating the palisade-tissue, and occasionally extending so far as to divide the latter into two portions. The chlorophyll-tissue, consisting of palisade-cells, is restricted to the ridges, and is formed of two or three layers of radially-elongated cells. This tissue is bounded on the inner side by a rather indefinite parenchymatous layer, which, by the conspicuous suberization of the radial walls of most of its cells, proves to be an endodermis. By reference to Figs. 1, 2, and 4, it will be seen to have a sinuous course, coming close to the surface beneath the furrows and curving inwards beneath the palisade-tissue so as to touch the cortical bundles.

There is one cortical bundle opposite each ridge of the stem, only separated from the palisade by the endodermis. Each cortical bundle is very small, its xylem being represented by only a few spiral elements; the phloëm, on the other hand, being considerably larger, and extended on either side along the face of the palisade, thus tending to give the bundle

a wedge-like shape. The point of the wedge, containing the xylem, is directed towards the centre of the stem, the orientation of these bundles being normal. The phloëm sometimes has a few sclerenchymatous elements on its outer limit (Figs. 1, 2, 3, 4).

On each side of the cortical bundle, thick-walled elements occur which are specially distinguished from the surrounding cells by their lignified, pitted walls, and by absence of protoplasmic contents. As seen in different sections they vary in shape and size: when large, they are rendered conspicuous by their numerous simple pits, and their irregular or angular, but parenchymatous shape; the smaller ones are often difficult to make out, but the thickness of their walls usually reveals them, as well as the phloroglucin-test, which stains them a bright red, while leaving the adjacent cells entirely untouched. These elements, which evidently constitute a transfusion-tissue, connect the xylem of the bundle obliquely with the palisade-tissue. The endodermis is, in places, occasionally interrupted by them. Loew [1] seems to have been the first to notice these elements. He devotes some space to the description of them and their position in the ridge, mentioning their exact position as seen in a transverse section of *C. pumila*, with regard to the bundle and the other portions of the tissue (Figs. 1–6). We shall have more to say about this tissue in describing the longitudinal section. Parenchyma, consisting of rather large, empty-looking cells, constitutes the remainder of the cortex.

There is a normal endodermis present surrounding the central cylinder (Fig. 2), followed by a rather indefinite, probably pericyclic layer, one or two cells thick, which is sometimes sclerotic opposite the phloëm, and may at that point form a thick strand of sclerenchyma.

The pith and primary medullary rays consist of parenchyma fairly similar to that of the cortex, and, together, form a star-shaped mass. The cells are often pitted.

[1] Loc. cit.

In a radial longitudinal section of a young stem, passing through the median line of one of the small leaf-teeth, one notices a layer of palisade-cells on its outer or dorsal side, which stops short at a little distance from the apex. On the inner side of this tissue there is a small bundle. The leaf-teeth are 'fused' below to form a short sheath, closely applied to the stem, this structure being precisely analogous to that of a gamopetalous corolla ending in a number of free segments (Fig. 7). Each leaf-tooth is continued downwards into a prominent ridge which extends to the next node below. This structure is regarded by various authors as an adherent leaf, Loew giving it the special name of 'phyllichnium,' while Lecomte prefers to call it simply a leaf.

In a longitudinal section made through one of these ridges, the transfusion-tracheides described above as occurring on the edge of the cortical bundle, are easily distinguished. In longitudinal section the character and function of these elements is much more clearly seen than in the transverse section. Owing to their position and the course they take, which is usually in an obliquely-ascending direction from the xylem of the bundle to the palisade-tissue, they appear to best advantage in a section which is not quite radial. In such a section they are sometimes seen in connexion with the bundle, sometimes quite isolated. A good example of the former case, as illustrating their function and relative position, was seen in a section which showed two spiral elements of the xylem of the bundle; running out from these elements, among the parenchyma-cells, was an extremely narrow, elongated tracheide, bent outward in its middle, and at its extremities abutting on the spiral tracheides of the bundle. Where this element bent outward it was in conjunction with another similar tracheide which took a more or less sinuous, longitudinal course down the side of the innermost cells of the assimilating tissue. *Directly opposite the point* where these two narrow tracheides were in contact, and separated from them by a single layer of cells, was a vertical row of four parenchyma-shaped transfusion-elements (Fig. 8, *tf*). As

will be seen from the figure, they are different in shape from the cells immediately surrounding them, and seem to be pushing themselves by sliding-growth outward round the inner ends of the palisade-cells. A little further down in the same row is seen a very small representative of a transfusion-tracheide, which is rounded in shape and apparently quite isolated.

These tracheides are often seen very well in a section which has passed through the phloëm, and missed the xylem, or where, perhaps, only a single element or so of the xylem is seen. Such a case is that of Fig. 9. On the outer edge of the phloëm occur rather narrow, longitudinally-elongated cells, which perhaps represent the endodermis. In the upper part of the drawing some of the assimilating cells abut on this layer. Lower down, however, is an element of a somewhat similar shape to the cells composing this layer, but rather narrower, very much pitted, and developed as a tracheide: it runs in a longitudinal direction, touching this layer on the outside. From this tracheide about four others, which are much larger, run outwards in a very oblique direction, and with their pointed ends press and push themselves between the surrounding green cells. These green cells are probably situated on the inner side of the palisade-tissue somewhere near the base of the furrow. The tracheides which we have here figured are very typical representatives of this transfusion-tissue. They are rendered very conspicuous by their extremely thick walls and very numerous and closely-placed simple pits. The two last cases are taken from sections of *C. glauca*, Sieb. In a section through the cortex of *C. muricata*, Roxb., a straight, longitudinal row of these tracheides was observed, separated from the palisade-tissue by two layers of cells. Their tracheidal nature was seen, of course, by their lignified, pitted walls, and their absence of cell-contents, but also by the way in which they seemed to utilize every chance of obtruding themselves between the ends of the adjacent cells. In this place, however, their course was not at all oblique, and formed, probably, part of

an oblique series of tracheides stretching from the xylem to the palisade-tissue.

The tracheides of this transfusion-tissue appear in all cases to be a modification of the parenchymatous-tissue of the cortex; as proof of this it was noticed in one section that, in a mass of similarly-shaped, irregular elements, some were developed as tracheides, with thick and pitted walls, while others, scattered amongst them, were ordinary thin-walled parenchyma-cells; and moreover, in one or two places, half of such an element was thick-walled and pitted, and the other half thin-walled and unpitted.

The pits are always simple, but of various sizes and shapes: often small and oval in outline, at other times narrow and slit-like, and sometimes stretching right across the cell, so as at first to give it the appearance of having spiral thickenings.

In one isolated instance, a tracheide was observed far out amongst the palisade-cells.

Loew[1], in his description of these elements, calls them 'thickened cells of the leaf-parenchyma,' comparing them with reticulate parenchyma-cells in the petiole of *Cycas revoluta*, and in the parenchyma of the leaves of *Sanseviera guineensis* and others. He is quite uncertain as to whether they belong to the vascular bundle or to the surrounding parenchyma.

Lecomte[2] also thus shortly mentions the occurrence of pitted, thick-walled elements connected with the tracheides of the cortical bundle:—'Ces faisceaux dans un certain nombre d'espèces, mais surtout dans le *C. quadrivalvis*, Labill., se ramifient à droite et à gauche, et ces ramifications, d'ailleurs très courtes, vont se terminer de chaque côté dans le parenchyme. Les trachées viennent appuyer leurs extrémités contre le cloison de cellules un peu plus grandes que les autres, à membrane un peu épaissie, et munie de ponctuations simples.'

Cluster-crystals of calcium oxalate frequently occur in some of the palisade-cells as well as in those of the inner cortical tissue.

[1] Loc. cit., p. 13.

[2] Loc. cit., p. 313.

The cortical bundles run down from the leaf through one internode along the edge of the palisade-tissue, and at the next node they pass in and unite with the central cylinder[1]. Their xylem seems to consist of spiral elements only, which are very narrow. The elements of the phloëm are so narrow that it is difficult to distinguish between the separate elements of which it is composed.

An examination of the *apex* of the stem distinctly showed three merismatic layers extending round this region. But the initial cell or cells of each layer were not clearly distinguishable from the adjacent cells.

Anatomy of Older Stem.

The transverse section of an older stem shows the formation of periderm in a hypodermal position, beginning in the form of an arc round the base of the depressions, the ridges containing the palisade-cells still remaining in free connexion with the stem. At a little later stage the cork completely cuts off the ridges, whose cells now appear shrunk and shrivelled, and their contents brown and dead. Lecomte[2] says that in *Casuarina* the periderm in the lower part of the ridge is formed outside the cortical bundle; higher up it is seen dividing this bundle into two parts; still higher up it appears on the inner side of the bundle; the periderm-strand continuing thus its perpendicular course while the bundle passes obliquely outward. In the same transverse section the periderm may be seen in three different positions: either on the outside of a cortical bundle, passing through its centre, or again, on the inside. At some point in the internode the cortical bundle cuts through the periderm (as mentioned by Lecomte). This author, as we have already said, describes the assimilating ridges of the stem as leaves, and he compares their separation from the stem by periderm

[1] De Bary, Vergleich. Anat. d. Phan. und Farne, p. 267.
[2] Loc. cit., p. 316.

with the process which takes place in the fall of the leaf in autumn.

In a transverse section of a still older woody stem the phloëm at the periphery is seen to be composed of elements somewhat irregularly arranged, consisting of sieve-tubes with a rather narrow cavity, companion-cells (Fig. 11), and phloëm-parenchyma. Some of the parenchyma-cells contain crystals of calcium oxalate.

The cells of the pith are generally large, often pitted and lignified, thus probably assisting in the conduction of water. Many of the cells may contain starch.

A considerable portion of the xylem is taken up by the medullary rays. These are of two kinds: there are rays consisting of larger cells and from two to several layers in tangential thickness, and other, more inconspicuous rays, consisting usually of only one layer of cells. Goeppert[1] was the first to describe these rays, which have been sufficiently well studied by later authors. Both kinds of rays are very numerous, and their cells contain starch and crystals of calcium oxalate, &c.

Lying between the rays, in regular radial rows, are the fibrous tracheides, composing the main portion of the wood. They usually have very thick walls; but this character varies in different parts of the stem and in different species. As usually seen, they possess a wall about equal in thickness to the width of the cavity, and with one or two bordered pits connecting them with surrounding tracheal elements. In some species, zones of fibrous tracheides occur in which the walls have acquired such a thickness as entirely or almost entirely to obliterate the cell-cavity, while no pits of any kind are to be seen in the wall; these elements have thus the general appearance of fibres, such as are typical for the higher Dicotyledons. In the outer portion of the wood of *C. torulosa*, Ait., there were zones of elements more irregularly placed with regard to one another which had far more the

[1] Loc. cit., p. 749.

appearance of fibrous cells than of fibrous tracheides, for they were full of contents and had quite a thin wall; but these were fibrous tracheides not yet fully mature, which retained their protoplasmic contents till their growth was complete. When a fibrous tracheide borders on a medullary ray-cell, the common wall has a half-bordered pit.

The *vessels* occur scattered throughout the wood, not arranged in any definite radial rows. In the primary wood they have usually a much smaller cavity, sometimes quite narrow, which increases in diameter as one passes further out. On the whole, the vessels in *Casuarina* have a comparatively narrow cavity; in one species they seemed rather larger than in any of the other ones examined. The vessels are usually surrounded by narrow parenchymatous cells; sometimes they border directly on fibrous tracheides; occasionally, two vessels are in contact with one another or with ordinary tracheides. The vessels occur scattered amongst the fibrous tracheides, and they may often be seen disturbing the course of a medullary ray and causing it to bend some considerable way out of its path.

Annual rings are not distinguishable, and the vessels are uniformly distributed throughout the wood.

The most striking feature in the wood of *Casuarina* is the concentric bands of xylem-parenchyma connecting the medullary rays. These were noticed by Goeppert[1], and called by him 'concentric medullary rays.' They were the 'false annual rings' of other authors. By means of these bands the wood is abundantly furnished with parenchyma. Sanio[2] gives this tissue the name of 'metatracheal parenchyma,' to distinguish it from that immediately surrounding the vessels, which he terms 'paratracheal.' The concentric bands are not quite regular in their course, being often interrupted by the vessels, and also here and there fusing above and below with one another. They contain starch, &c. (Fig. 12 *pm*).

If a section is made through that part of the stem where

[1] Loc. cit. [2] Loc. cit., 1863, p. 389.

a branch is passing in, there will appear a gap in the central cylinder comprising about one-third of the whole section. This gap is filled with loose, parenchymatous tissue, dotted with large numbers of scattered tracheides with a tolerably thick wall. At the sides of the gap many of the elements can be seen joining on to the central cylinder, but the majority of them consist of the protoxylem-elements of the branch which are passing obliquely inwards to the centre of the stem. They give a characteristic appearance to the section. For some distance below the point where the tracheides have all united with the central cylinder of the stem the large ray thus formed is still present. These tracheides were described by Goeppert[1] and Lecomte[2]. The former figured them roughly in a tangential section of *C. torulosa*, Ait., but did not account for their appearance.

A longitudinal radial section of a *woody* stem of *Casuarina* shows the phloëm to consist of sieve-tubes with very oblique terminal walls on which several sieve-plates are scattered. These sieve-tubes are very narrow and the companion-cells are not easy to distinguish (Figs. 13–15).

Most of the medullary rays in the wood are several cells, some only one or two cells thick. The most common form of ray consists of square, very thick-walled cells, with simple pits in their lignified walls, and often copious contents of starch, &c. In one or two cases a bordered pit was noticed in a wall of one of these cells, which was void of contents, thus constituting it a tracheide. In some species there were two kinds of rays: those above described, and others with thinner-walled cells which are narrower and elongated radially.

Parenchymatous tissue amongst the elements of the wood is abundant. The cells composing it are of various shapes and sizes; they are sometimes short and narrow, at other times broader, or much more elongated. Their walls are often considerably thickened. They invariably have contents. The more elongated cells of this tissue, which undergo no

[1] Loc. cit. [2] Loc. cit., p. 315.

subdivision by transverse walls in the cambium-segment, were given by Sanio[1] the special name of 'Ersatzfasern.' Special cells are also set apart for storing up crystals of calcium oxalate; these cells are distinguished by their small size, rectangular shape, and their arrangement in longitudinal rows; they each contain usually a single crystal of about the same shape as the cell itself, sometimes, however, a cluster-crystal. All the elements thus far described belong to the concentric bands which are seen in transverse section (Figs. 17, 28). The parenchyma-cells surrounding the vessels may vary in shape and size as do those in other parts of the wood; but there is nothing abnormal about them. A cambium-segment has in most cases divided so as to form a vertical row of four parenchyma-cells; the end-cells of these rows have often undergone slight sliding-growth, their pointed ends being intruded between surrounding cells. Where the walls of these elements border on a vessel, they are, of course, thickly covered with bordered pits; when they abut on one another, however, the walls have simple pits (Fig. 16).

The *vessels*, as seen in this section, vary considerably in appearance, both as regards size and the mode of perforation of their terminal walls; these latter are always more or less oblique. In the primary wood, i.e. the first-formed after the protoxylem, the vessels are usually the narrowest, and as a rule become wider as they get farther out in the secondary wood. This plant is an instance of the occurrence in the wood of vessels with a single perforation, and of those with several perforations, with all transitions between the two. As a general rule, the typical vessels of the secondary wood possess wide cavities, and a single, large, round, or oval perforation in their terminal wall, which latter is more or less oblique, being inclined to the radial plane, so that its surface is clearly seen in a longitudinal radial section. The lateral walls of the vessel are thickly covered with bordered pits.

[1] Loc. cit., p. 96.

In some species this type of vessel clearly predominates (Figs. 19, 25). In others, however, the single perforation is, in the majority of elements, replaced by a large number, often as many as twelve, of ladder- or slit-like perforations, which give a very characteristic appearance to these elements; in these cases the round or oval form of the wall remains unchanged. In such a stem as, e.g., that of a doubtfully-named species which we examined, one may find all transitions from these ladder-like perforations to the single, round perforation above-mentioned; from the complex network shown in one vessel in the plant mentioned, and figured in the drawing, the transverse bars gradually become fewer and fewer in number and the perforations between them correspondingly wider, till, as seen in some elements, only three, two, or even one flimsy bar stretches across, or only part way across, the perforation (Figs. 20–22).

In many cases tertiary spirals occur in the vessels; these are often very conspicuous, and sometimes double.

In the primary wood, however, we get, as it were, quite a different series of vessels, though many of the forms found here also occur here and there in the secondary wood. In one instance, shown in *C. muricata*, Roxb., quite near the protoxylem, were some very narrow elements with extremely oblique terminal walls, on which was a row of small, irregularly-shaped perforations, precisely identical with those to be described and figured later on for *Carpinus*. Another case showed a narrow vessel with *three small, round* perforations, placed at short distances from one another on a very oblique terminal wall. As showing what would appear as the result of the fusion of the several perforations in the two latter cases, elements frequently occurred, usually slightly further on the outside, with a single, long, narrow perforation, at whose edges slight projections were seen, indicating where the transverse bars in an earlier formed element would have stretched across. Fig. 18 also shows a case of an element from the primary wood. One more case worth mentioning, as forming a connecting link with the tracheides, was that of a terminal

wall of a vessel having at each end bordered pits, while in the centre were one to two small perforations.

As evidence of the great amount of sliding-growth taking place between the ends of the vessels, macerated material showed a long, pointed piece of wall extending a long way past the locality of the perforation (Fig. 25).

The *fibrous tracheides* form the great bulk of the wood, and, as already described as seen in transverse section, they vary considerably in appearance. The type which is perhaps of the most frequent occurrence has a cell-wall about equal in thickness to the width of the cavity, with conspicuous bordered pits at short intervals from one another, the cavity of the pit being fairly large. The outline of the pit is usually circular, and the opening is slit-like and oblique, so the superposed openings on each side of the common wall often give the appearance of a cross (Fig. 26). In other parts of the stem, however, of the same or of another species, broad zones occur, consisting entirely of fibrous tracheides whose walls have become excessively thickened, so much so as to reduce the width of the cell-cavity to a minimum, while the wall itself is, in many cases, split rather widely apart at the middle lamella, so that at first this gives the false appearance of a cell-cavity. In these elements no pits of any kind are to be seen; but we may presume that they are of the same nature and origin as the above-mentioned fibrous tracheides, and that during elongation of the element, and the process of thickening of the cell-wall, the rudiments of the bordered pits, which most likely had begun to be formed, soon become entirely suppressed and obliterated. This is the more probable, as in other parts of the stem elements were found with a wall of considerable thickness, but in which bordered pits occurred, though of very small size, and at distant intervals from each other; they were in process of becoming suppressed like those in the more highly differentiated elements[1] (Fig. 27). The fibrous tracheides have bordered pits on all their walls;

[1] Solereder, loc. cit.

where they abut on one another or on vessels or tracheides there are always bordered pits on the common wall; where they abut on parenchyma-cells, however, there is always a half-bordered pit on the common wall. Strasburger[1] and Sanio[2], in writing of other cases investigated by them, cite the two facts of the occurrence of bordered pits on the wall separating two 'fibrous tracheides,' and the formation of parenchyma among these elements as evidence of their tracheidal nature. The occasional occurrence of a tertiary spiral in these elements points to the same conclusion.

Ordinary tracheides likewise occur, though they are few in comparison with the other elements of the wood: Fig. 28 shows one near the pith; on its outside is a row of parenchymatous elements, which in their turn abut on a fibrous tracheide. Transitional forms between the tracheides and the fibrous tracheides occur.

In one unnamed species a very interesting form occurred, which exhibited very clearly the mode in which the slit-like perforations of the vessels have their origin: at each end of the terminal wall of the tracheide bordered pits were irregularly scattered; but towards the centre of the wall they gradually grouped themselves in transverse rows of two or three, at first unconnected with each other, afterwards partially united, till, finally, what appeared as one large slit-like bordered pit was formed by the complete fusion of two or three small ones (Fig. 29).

In some sections made on that side of the stem, where a branch had lately passed in, a longitudinal series of tracheides was observed, which were running irregularly down in the broad zone of parenchymatous tissue described above[3]. They possessed dense spirals and pointed ends, and were evidently protoxylem elements. These are the same elements which have been described above as seen in transverse section. As we have also seen, they were mentioned

[1] Ueber den Bau und die Verricht. der Leitungsb. in den Pflanzen, 1891.
[2] Loc. cit., 1863, pp. 115, 116.
[3] Above, p. 243.

by Goeppert and Lecomte; but we should also add that Sanio[1] describes the same thing. They may evidently be defined as the protoxylem elements of the branch which has united with the stem above, and they are pursuing, by a very oblique course, their way to the centre of the stem, there to unite with its protoxylem. These tracheides present a unique appearance, passing thus isolated through the parenchyma for such a distance through the stem. The elements of the secondary wood of the branch pass off right and left of these tracheides to unite with the secondary wood of the central cylinder of the stem, while the large ray thus formed unites the pith of the two organs (Fig. 30).

In the cortex of the stem of *Casuarina*, on its innermost side, next to the phloëm, a longitudinal series of large stone-cells regularly occur; they are oval or round in shape, and have very thick walls with simple pits running through them. Other cortical cells contain, like those of the medullary rays and parenchyma in the wood, crystals of calcium oxalate, which are usually single rhomboidal ones. In a young stem of *C. stricta*, Ait., a large part of the cortex was taken up by sclerenchyma fibres.

Anatomy of Root.

The structure of the young *root* is *tetrach*. This character very soon becomes difficult to make out, owing to the early appearance of secondary thickening, which produces a uniform structure of wood in this organ. In the older root the most striking feature is the large size of the medullary rays and the abundance of starch which they contain. The amount of woody tissue present is often reduced to a minimum, the greater part of the root being built up of parenchymatous tissue containing an immense store of large starch-grains. In the case of a root of *C. torulosa*, Ait., examined, the structure consisted of six triangular zones of tissue as seen in transverse section; three of these zones consisted of woody tissue, and

[1] Loc. cit., p. 127.

three of them of parenchyma, these alternating the one with the other; half of the whole circumference of the root was thus taken up by the three broad medullary rays, whose cells were thin-walled, more or less elongated radially, and densely packed with starch-grains.

Remarks on the Structure of the Stem of Gnetum and Ephedra.

As the Gnetaceae are regarded as the highest of the Gymnosperms, *Gnetum* and *Ephedra* were examined for comparison with *Casuarina*.

Some structural points in *Gnetum* and *Ephedra* are of independent interest, and will therefore be described at some length. The species of *Gnetum* examined were—*G. Gnemon*, L., *G. paniculatum*, Benth., *G. scandens*, Roxb., *G. neglectum*, Blume, *G. Thoa*, R. Br.

In these species the vessels formed next after the protoxylem mostly have oblique end-walls with several round perforations or a single, long, narrow one. The later-formed vessels differ in having a single round perforation in each terminal wall. In the later vessels of *G. Gnemon*, most of the end-walls have a row of round perforations, while some have one long, narrow perforation, which would result from the fusion of several of the round ones (Fig. 31). Tracheides occur with a row of large, round-bordered pits on the terminal walls, as in *Ephedra*. One vessel in *G. scandens* showed an extraordinary long, oblique end-wall with many round perforations (Fig. 32). In *Gnetum Thoa* the first vessels after the protoxylem have one to two large (Fig. 35), or several round perforations. *Gnetum paniculatum* was the most interesting species examined, as the node differs considerably from the internode in structure; the node agreeing with *Ephedra* in the perforation of the vessels, &c. In the internode the first-formed vessels have several round perforations (Fig. 36); the outer ones have a single perforation. In one

place, next to the protoxylem, was a vessel with a single, long, narrow perforation, while one or two elements farther out was one with several perforations. The structure of the *node* in every point resembles that of *Ephedra* to a degree which is very striking to one accustomed only to the ordinary structure of the internode. There are tracheides with two conspicuous rows of bordered pits on their terminal walls, these pits having precisely the appearance of those which are characteristic of *Ephedra*. Among these occur vessels whose lateral walls are covered with the large round bordered pits, while their terminal walls are precisely analogous to *Ephedra* in every respect, having, like that plant, *two distinct rows of small, round perforations*, each one the size of the bordered pit from which it is derived. These vessels are also narrower than those in the internode. As seen in the stem of *Ephedra*, transitions also occurred here between tracheides and vessels, some of the perforations having more or less of a border round them, others having no border remaining (Fig. 37) (cf. Fig. 41). On some terminal walls there were perforations in the centre and bordered pits at each end. As you get lower down towards the internode the structure *gradually* passes over into that typical for *Gnetum*: the opposite, adjacent perforations on the terminal walls of the vessels are seen to fuse two and two to form a single perforation, this resulting in a single row of somewhat oval perforations considerably larger than the original ones of the node; this stage was observed here and there, as represented by Fig. 38, which is, however, taken from the stem of *G. Gnemon*, L.; farther down still the perforations thus formed fuse with those above and below to form the single, narrow, long perforation when the fusion is complete; all transitions between it and the first stage occur where the fusion is irregular; as in some instances the bordered pits are often placed very irregularly on a rather broad terminal wall, the result of fusion is a rather complex arrangement of perforations. Usually the perforation is not so complete, but that a distinct border is still left round it (Figs. 39, 40). The same kind of fusion is seen in

the first-formed vessels of a young stem of *G. Gnemon*, where a double row fuses to form a single row of perforations, as shown in Fig. 38, while most of the other vessels in the wood have a row of round perforations, some, however, showing *one* long, narrow perforation, marking thus a further higher modification in the fusion of several to form one perforation.

Short-celled parenchyma occurs round the vessels in the wood of *Gnetum*. It is characterized by having thick walls. Sections of the wood of some were treated with iodine-solution, but no starch was seen, though there was abundance of it in the pith.

The phloëm of *G. paniculatum*, Benth., *G. Thoa*, R. Br., *G. neglectum*, Blume, and an unnamed Sumatran species, showed the following:—sieve-tubes with oblique terminal walls; compound sieve-plates on these and on the lateral walls. In some the sieve-plates on both walls were clearly seen without staining. The albuminous cells were very distinct in some species; they formed regular rows alternating with the rows of sieve-tubes, and occurred in the same radial line with the medullary rays in the wood. Though they sometimes look extremely like companion-cells, especially when, through the crushing of the elements, they come to lose their regular position and lie at the corners of the sieve-tubes, they are apparently formed from the same cambium-cell which cuts off the medullary ray-cells in the wood. Seen in longitudinal section, the albuminous cells are narrow, short cells, filled with dense, proteid contents.

In the cortex are many greatly elongated brown fibres, and stone-cells similar to those seen in *Casuarina* and the Cupuliferae.

The protoxylem of *Gnetum* consists of elements with a dense spiral or reticulate thickening, amongst which bordered pits occur at intervals. In a few instances these pits appeared to be resolved into round perforations.

In *G. neglectum* there occur, scattered through the wood, groups of much-elongated, thick-walled elements, with cell-

contents. They are identical in nature with those which are known to occur in *Ephedra*.

The stems of *G. Thoa* and *G. scandens* which we examined were too young to show the anomalous secondary thickening described by De Bary[1] and Strasburger[2], but the Sumatran species showed one anomalous zone of xylem and phloëm.

Apparently *Gnetum* has hitherto been described as possessing vessels with *one* perforation only, and Strasburger[3] mentions this as one of the features which distinguish it from *Ephedra*. The vessels first formed by the cambium (in the internode) seem to have been overlooked; they almost invariably have several perforations.

The anatomy of *Ephedra* is sufficiently well known; but some of the points in which it differs from and agrees with *Gnetum* might be mentioned.

One striking feature is the constant occurrence throughout the secondary wood of vessels, some with a double, some with a single row of perforations on their terminal wall. Besides vessels there are also corresponding tracheides. They differ from the vessels in having bordered pits instead of perforations in their terminal walls, the former being just the same size as the last, and in there being usually one row of these instead of two, as in most of the vessels. But the most interesting part of this wood-structure consists of the transitional forms which are everywhere seen between tracheides and vessels. On some walls the central apertures were true perforations, while those towards the ends were bordered pits (Fig. 41). Some vessels were seen in which some of the perforations had still part of the border left round them, these having, often, on one side of them bordered pits, on the other thoroughgoing perforations. Indeed, almost all transitions are seen, where the border becomes narrower and narrower, till, finally, both pit-membrane and border disappear, and a true perforation is formed. Besides these elements there is the characteristic parenchyma of the wood, consisting of much

[1] Loc. cit., p. 603 (Fig. 233). [2] Loc. cit., p. 147.
[3] Loc. cit., p. 144.

elongated, fibrous elements with granular contents and starch, which Sanio [1] mentioned long ago under the name of 'Libriformzellen' as constituting one of the chief differences between the anatomy of this plant and that of the Coniferae; they are probably formed from cambium-segments which, after being cut off, undergo no transverse division whatever. The cambium-cells themselves appeared to be of great length. These elements are analogous to those in *Gnetum* (described above).

The phloëm is much the same as in *Gnetum*; the elements composing it are approximately of the same diameter. The sieve-tubes have very oblique terminal walls with compound sieve-plates, these latter also occurring on the lateral radial walls. The albuminous cells adjoining the sieve-tubes have dense contents with large and long nuclei, some of which appear curiously constricted in the middle. There are very numerous brown fibres in the cortex.

The anatomy of the node of *Ephedra* is very different in many respects from that of the internode. On looking at either a transverse or a longitudinal section of this part of the stem, one is at once struck with the uniformity of the structure, all the elements being approximately of the same size; the characteristic vessels of the internode are entirely absent, and the tracheal tissue consists entirely of tracheides; in fact, the wood here resembles very much that of *Taxus* or some other Taxaceous genus, owing to the complete absence both of the vessels and of the wider tracheides. Another peculiarity of this part of the stem lies in the striate appearance of most of the tracheides; these striae, which usually form a double spiral round the element, are very faint, and in no way comparable to the tertiary spirals seen in *Taxus*, *Casuarina*, &c.; in many cases it was plainly seen that this appearance was due to a regular splitting of the wall, as the faint lines were continuous with the slit-like openings of the bordered pits, and formed often quite wide slits at these points. In other

[1] Loc. cit., 1863, p. 406.

elements, where the spiral lines were much fainter and much closer together, they did not give this appearance, and in such cases it was not easy to say what they really were. Similar spiral lines were also noticed in the tracheides of *Podocarpus* and *Phyllocladus*. Also the bordered pits of the elements in the node differed from those in the internode in the fact that their openings were much wider and more slit-like.

In examining a young stem of *Ephedra* in which the bundles were still separated by parenchyma, transfusion-tissue was observed in the medullary ray, connecting the xylem of the bundle with the innermost cells of the palisade-tissue. They were tracheides, possessing both reticulate thickenings and bordered pits, and had no contents. They were scattered promiscuously amongst the parenchyma-cells, whose shape they still retained for the most part, though some were considerably more elongated longitudinally. Sometimes an uninterrupted row of them extended down through the tissue. They were elements somewhat of the same nature as those forming the protoxylem of *Gnetum* and *Ephedra*, and it is the same type of tissue, though better developed, as occurs on either side of the bundle in the leaf of *Ephedra* (Fig. 42).

In conclusion, it may be pointed out that *Ephedra* and *Casuarina* both have rudimentary leaves, assimilating stems, and transfusion-tissue; but while the transfusion-tissue of *Ephedra* has bordered pits, that of *Casuarina* has only simple pits. This may mean that the transfusion-tissue of *Casuarina* is only a recent transformation of ordinary parenchyma.

Again, the structure of *Gnetum paniculatum*, described above, might be interpreted as showing a tendency to reproduce an ancestral type of structure at the node; especially when compared with the nodal structure (cambium) described by Cormack[1] in *Equisetum*. It must, however, be remembered that the requirements for the conduction of water at the node, as also the mechanical conditions there, are no

[1] Cormack, Annals of Botany, 1893.

doubt different from those in the internode, and may determine the type of element produced. The researches of Prunet[1] illustrate this point, for he observed that, in certain plants showing a distinction between nodal and internodal structure, this distinction does not occur in the underground part of the stem.

Remarks on the Structure of the Stem in the Cupuliferae.

A glance at the structure of some members of the Cupuliferae, which indeed show some affinity in their anatomy with *Casuarina*, will be of interest. Taking just four genera for this purpose, we see that though there is diversity of structure in each of these, there is considerable agreement in the ground-plan.

Looking at *Quercus* first of all, we find this plant, in its general structure, to bear considerable resemblance to *Casuarina*. The vessels with a tendency to form several perforations are found chiefly near the protoxylem. Most of the others have a single perforation. There are tracheides, and transitions from these to vessels. Fibrous tracheides form the bulk of the wood. The parenchyma is very well developed.

In *Fagus* the vessels have both one and several perforations, the former the most frequent. Strasburger[2] states with regard to the end-walls of the vessels that the least oblique have a *single* perforation, the more oblique a ladder-like or reticulate perforation; while there are some with both bordered pits and perforations, and others with bordered pits only. As in *Quercus*, there are short, oblique walls in the sieve-tubes of one to three plates.

Carpinus Betulus, L., has vessels with both kinds of perforations. The great majority, however, are of the ordinary dicotyledonous type, with a single perforation. But next to

[1] Prunet, Ann. Sci. Nat. Bot., 7 sér. tome XIII, 1891, p. 297.
[2] Loc. cit., p. 273.

the protoxylem, narrower vessels were found with many perforations on their end-walls. These perforations were extremely interesting, for they were of very irregular shapes, this being due to the fusion of the bordered pits in twos and threes, or more, as they lay irregularly scattered on the wall, to form the perforations, fusing sometimes at an oblique angle, sometimes horizontally, or both combined (Fig. 43). In some cases the narrow extremity of a perforation was occupied by one of these bordered pits still unabsorbed, but forming a continuous portion of the perforation (Fig. 44). We also saw cases of the fusion of these perforations with those above and below, thus indicating, how from several perforations, one single narrow oblique perforation is eventually attained (Fig. 43). These vessels are precisely similar to those described above in *Casuarina muricata*, Roxb. The bordered pits of the vessels differ somewhat from those of the other genera in being perfectly round in outline, with a circular opening, giving a characteristic appearance. The fibrous tracheides here are similar to those in some of the others; the borders of the pits are little developed, and the wall of the element is not so very much thickened. In the sieve-tubes are numerous plates on a very oblique wall.

In *Carpinus cordata*, Blume, the structure of the wood differs considerably from that of *C. Betulus*, L. Indeed the structure is almost a reproduction of that of *Corylus Avellana*, L. All the vessels have several perforations, resembling in every respect those in *Corylus*. This plant agrees better in its anatomy with *Corylus* than with *Carpinus Betulus*, L.

Corylus Avellana, L., showed vessels which had terminal walls with exclusively *several* perforations. There are also tracheides. An extremely interesting transition was observed on the terminal wall of one of these latter: at each end was a great number of closely-placed bordered pits; towards the centre these were seen fusing in twos and threes, forming a few elongated, slit-like bordered pits (Fig. 45). It will be seen that this is a transition from a tracheide to a vessel.

A similar case has been described in that part of the paper dealing with *Casuarina*.

Structure of the Seedling.

The seedlings of *Ephedra*, *Gnetum*, and *Casuarina* show great similarity of structure. The seedling has two cotyledons, the number varying in the two latter genera sometimes to three, as occasionally happens in several Dicotyledons. Alternating with the cotyledons, higher up on the stem, are two plumular leaves; these may have branches in their axils; the cotyledons also have branches in their axils; and there are often secondary axillary branches inserted between the primary ones and the cotyledons. These points are common to all three genera.

A transverse section through the lowest internode of the plumular stem of *Casuarina*[1] will show eight, or sometimes more, central bundles, which have arisen by the splitting of each of the four bundles which have passed into the centre from the later-formed leaves above. A little lower down these eight bundles fuse again in twos, and four central bundles as before is the result. This splitting up of the central bundles is also described as occurring in the seedling of *Gnetum*[2]. Where the section passes through adherent portions of the two first plumular leaves and the two cotyledons, a more complex system of bundles will be shown. The bundle of each of the two first leaves will appear some way out. Between each of these bundles and the central bundles of the plumular stem will be seen two bundles, which evidently belong to a branch in the axil of the leaf. Then alternating with the position of the leaves, and still farther out, will be about four bundles coming from each cotyledon, and between these and the central bundles of the stem on each side, two large bundles from an axillary branch. Still further down, at the median point of insertion of the cotyledons, we see four central bundles as before, the bundle

[1] Morini, Anatomia del Frutto delle Casuarinee, 1890.
[2] Bower, Quarterly Journ. Microsc. Science, 1882, p. 287.

from each of the two first leaves in the act of passing into the centre, and, alternating with these, two bundles from each cotyledon coming in to join the four central bundles. These two cotyledonary bundles may either remain as two separate though closely-placed bundles, or they may fuse into one; as they pass in together their protoxylems seem to turn towards each other. A little below, the transition from stem to root takes place.

As far as could be made out, this seems to follow the dicotyledonous mode; the four bundles from the plumular stem fuse together in twos, their xylems severally 'rotating'; the two bundles from the cotyledons, each of which represents really two distinct bundles, remain as they are, their xylems simply 'rotating'; the phloëm, however, separates, and each half fuses with the phloëm of the plumular bundle on each side. So that thus a tetrarch root is formed, with four primary xylem-, and four primary phloëm-groups.

In *Gnetum* there is a difference; according to Bower[1] the xylem of only two of the bundles rotates on its axis, while the other bundles, of which there seem to be several, do not rotate, but pass down and finally become lost in the cambium of the root. The phloëm of each of the rotating bundles divides into two, which mutually fuse to form the primary phloëm; this corresponds with the two cotyledonary bundles in *Casuarina*, whose phloëm divides in the same manner.

In *Casuarina*, the endodermis appears just after the cotyledonary bundles have passed in, and there is a pericycle of two to three layers. Cuticularization in the endodermis also extends to the tangential walls, as in *Gnetum*.

SUMMARY.

The result of our anatomical investigations confirms what previous authors have stated as to the rather isolated position which *Casuarina* holds among Dicotyledons, by reason of the

[1] Loc. cit., p. 285.

following peculiarities of its stem-structure: (*a*) the great development of xylem-parenchyma in the form of concentric bands; (*b*) the very broad medullary rays (sometimes occupying one-third of the circumference of the stem) as well as narrow ones one cell thick. These very broad ones are connected with the passing in of the bundles of a branch; the latter, by their very oblique course inwards, give a characteristic appearance to the stem. Such characters as these, however, do not appear capable of leading to any important systematic conclusions.

Of the more detailed characters, the tracheal part of the xylem, consisting of vessels with both numerous and single perforations, together with fibrous tracheides, which are largely fibre-like in appearance, resembles that of the Cupuliferae and other lower forms of Dicotyledons, as also a few higher ones.

A point which has not been fully noticed by former writers is the following:—that the vessels in the primary and early secondary wood are different from those in the later-formed wood, and show characters of a preceding type of structure. The fibrous tracheides appear to be originally derived from tracheides such as constitute the bulk of the wood of the Gymnosperms, and seem to precede the type of fibre-tissue of the higher Dicotyledons, which is probably parenchymatous in origin [1].

The phloëm is dicotyledonous in structure.

Transfusion-elements occur in the ridges of the young stem and in the leaves. They have evidently a distinctly different origin and nature from the transfusion-tissue so well known in the leaves of Gymnosperms, as is shown by their possessing simple pits and no reticulate or spiral thickening; they are adapted to the position and orientation of the cortical bundle in the stem with regard to the assimilating tissue of the ridge, and to the peculiar structure of the leaf-sheath.

Another feature, which we think has not been noticed

[1] Strasburger, loc. cit.

heretofore, is the presence in the young stem of an *external endodermis* extending round the outer limit of the cortical bundles and dipping beneath the furrows; its presence was plainly shown on treatment of the section with strong sulphuric acid.

The characteristic formation of periderm in this plant has been fully described by former authors.

With regard to the structure of the seedling we have only to mention that in the main it agrees with the dicotyledonous type.

It is a matter of interest to consider whether the anatomy of *Casuarina* affords evidence, favourable or unfavourable, bearing upon the systematic position assigned to the genus by Treub[1]. In the structure of its phloëm, *Casuarina* shows no important departure from the dicotyledonous type, and in its xylem-elements it agrees pretty well with the Cupuliferae and other Dicotyledons. In the disposition of its xylem-parenchyma, &c., it forms a rather isolated case, but this would not appear to be of any fundamental importance. On the other hand, the resemblance of the wood-structure in *Casuarina* to that of the Cupuliferae is of some importance, in the light of the researches of Miss M. Benson[2] on the reproduction of the Amentiferae: but this importance is much diminished by one or other of the characters of agreement occurring in the wood of Rosaceae, Saxifragaceae, Cornaceae, &c.

The chief result of our examination of *Gnetum* has been the establishment of the fact that the wood possesses two kinds of vessels, viz. those of the primary and early secondary wood, with several perforations, or with a long, narrow, single perforation, and those so well known in the secondary wood, with a single round perforation.

Still more important, perhaps, is the structure of the node,

[1] Treub, Sur les Casuarinées et leur place dans le Syst. Nat., Annales du Jard. Bot. de Buitenzorg, X, p. 145.

[2] Benson, Contributions to the Embryology of the Amentiferae, Linn. Soc. Trans., 1894.

which is so entirely different from that of the internode; instead of the typical *Gnetum*-structure we found elements (vessels and tracheides) possessing characters intermediate between *Ephedra* and *Gnetum*, the mode of perforation of the vessels more nearly resembling that in the former plant than the latter; we have fully entered into the description of this phenomenon in the foregoing pages.

The above investigation was first begun at the suggestion of Dr. D. H. Scott, and has been carried out in the Botanical Laboratory of the Royal College of Science, South Kensington. The examination of the stem and leaf-sheath was begun by L. A. Boodle; the completion of this, together with the remaining part of the paper, was carried out by W. C. Worsdell. We wish to express our thanks to Dr. D. H. Scott and Prof. J. B. Farmer for their kind help and suggestions, during the earlier and later parts of the work respectively.

In conclusion we must particularly mention our indebtedness to Mr. Carruthers and to Mr. Rendle of the Natural History Museum, for having given us facilities for examining dried material of several species of *Gnetum* in the Herbarium.

We have also to thank the Director of the Royal Gardens, Kew, for kindly supplying us with living specimens of various species of *Casuarina*.

EXPLANATION OF FIGURES IN PLATES XV AND XVI.

Illustrating Messrs. Boodle and Worsdell's paper on the Comparative Anatomy of *Casuarina*.

Fig. 1. Portion of a transverse section of a young stem of *C. glauca*, Sieb., showing the cortical bundles (*ctb*), the transfusion-tissue (*tf*), and the palisade-tissue of the ridge (*ps*), with the outer protective layer of sclerenchyma (*sc*). On the inside is the central cylinder, xylem (*x*); phloëm (*ph*); pith (*p*). × 145.

Fig. 2. Portion of a transverse section of a young stem of *C. glauca*, Sieb., showing the external endodermis (e^2) running beneath the palisade-tissue (*ps*) and on the outer limit of the cortical bundle (*ctb*). On the inside is a single bundle of the central cylinder (*b*) with the internal normal endodermis (e^1) extending round it. *ct* = cells of the cortex. × 500.

Fig. 3. Transverse section through a cortical bundle of *C. glauca*, Sieb., showing the relative amount of its xylem (*x*) and phloëm (*ph*) and the groups of transfusion-elements (*tf*) running out from the side. × 500.

Fig. 4. Transverse section through a cortical bundle of *C. stricta*, Ait., showing the group of sclerenchyma-fibres (*sc*) on the outer limit of the phloëm, a large transfusion-tracheide (*tf*) with a portion of the endodermis (e^2) running round it. × 335.

Fig. 5. Transverse section through a cortical bundle of *C. glauca*, Sieb., showing transfusion-elements. × 500.

Fig. 6 *a*. Transverse section of a transfusion-tracheide of *C. stricta*, Ait. *sc*, sclerenchyma. × 500.

Fig. 6 *b*. Transverse section of a transfusion-tracheide of *C. glauca*, Sieb.

Fig. 7. Portion of a transverse section through the leaf-sheath of *C glauca*, Sieb. × 145.

Fig. 8. Longitudinal section through part of the xylem (*x*) of the cortical bundle of *C. glauca*, Sieb., showing two long narrow tracheides running out to the transfusion-tissue (*tf*). × 335.

Fig. 9. Longitudinal section through part of the phloëm (*ph*) of the cortical bundle of *C. glauca*, Sieb. A small bit of one of the tracheides of the bundle is shown (*x*). e^3, the probable endodermal layer; *tf*, the transfusion-tissue; *ps*, the innermost cells of the assimilating tissue lining the furrow. × 500.

Fig. 10. Longitudinal section which has passed to one side of the cortical bundle, and shown a row of transfusion-elements abutting on the face of the palisade-tissue (*C. glauca*). × 500.

Fig. 11. Transverse section of sieve-tubes and their companion-cells from the phloëm of *C. sp.* × 500.

Fig. 12. Transverse section through a portion of the wood of *C. glauca*, Sieb., showing three transverse bands of parenchyma (*pm*) traversing the fibrous tracheides (*f. tr*) between two medullary rays *mr*). Each of the bands forms part of a concentric circle of tissue or 'false annual ring.' × 335.

Fig. 13. Longitudinal sections through the terminal walls of three sieve-tubes of *C. glauca*, Sieb., one of which is radial, showing four plates in surface-view, the other two tangential, showing from two to eight plates in section. × 500.

Fig. 14. Longitudinal radial section of terminal wall of a sieve-tube of *C. glauca*, Sieb., showing nine plates in surface-view. × 335.

Fig. 15. Longitudinal section of a portion of a sieve-tube of *C. glauca*, Sieb., showing a companion-cell. × 500.

Fig. 16. Longitudinal section of parenchymatous tissue of *C. tenuissima*, showing a vertical row of four parenchyma-cells abutting on a vessel. × 250.

Fig. 17. Longitudinal section of two cells from a concentric band of parenchymatous tissue of *C. glauca*, Sieb. × 335.

Fig. 18. Terminal wall of a vessel from a radial section of *C. sp.*, showing a single narrow perforation. × 250.

Fig. 19. Longitudinal section of a portion of a vessel of *C. glauca*, Sieb., showing the terminal wall, with a single large perforation, in section. × 250.

Fig. 20. Terminal wall of a vessel in *C. sp.*, exhibiting ladder-like perforations. × 250.

Fig. 21. Terminal wall of a vessel in *C. sp.*, showing reticulate perforations. × 500.

Fig. 22. Terminal wall of a vessel in *C. sp.*, showing transitional stage from ladder-like or reticulate to a single perforation. × 250.

Fig. 23. Terminal wall of a vessel in *C. glauca*, Sieb., showing ladder-like perforations in section. × 335.

Fig. 24. Vessel from the wood of *C. suberosa*, Ott. et Dietr., showing a tertiary spiral. × 335.

Fig. 25. Vessel from the macerated wood of *C. glauca*, Sieb., showing the narrow prolongation beyond the position of the perforation. × 250.

Fig. 26. Portion of a fibrous tracheide from the wood of *C. sp.*, showing its closed end, and the bordered pits on the walls. × 250.

Fig. 27. Portion of a fibrous tracheide from the wood of *C. glauca*, Sieb., in which bordered pits have become entirely suppressed. × 250.

Fig. 28. A tracheide occurring near the pith; with a row of parenchymatous elements and a fibrous tracheide on its outside (from *C. glauca*, Sieb.). × 250.

Fig. 29. Terminal wall of a tracheide from the wood of *C. sp.*, showing transition from bordered pits to ladder-like perforations. × 250.

Fig. 30. Longitudinal section through the large medullary ray in *C. glauca*, Sieb., showing the protoxylem-elements of the branch passing obliquely downwards to the pith. × 335.

Fig. 31. Terminal wall of a vessel from the young stem of *Gnetum Gnemon*, L., showing a single narrow perforation. × 250.

Fig. 32. Terminal wall of a vessel from the primary wood of *G. scandens*, Roxb., showing a row of eight round perforations. × 165.

Fig. 33. Terminal wall of a sieve-tube from the phloëm of *Gnetum scandens*, Roxb., showing a large number of sieve-plates scattered over it. × 250.

Fig. 34. Portion of a sieve-tube from the same plant, showing the region of the sieve-plates. × 120.

Fig. 35. Terminal wall of a vessel from the primary wood of *G. Thoa*, R. Br., showing a long narrow perforation still divided into two by a transverse wall. × 165.

Fig. 36. Terminal wall of a vessel from the internode of *G. paniculatum*, Benth., showing three perforations, of which the upper one can be clearly seen to be derivative from two. This is an earlier stage than Fig. 35. × 250.

Fig. 37. Terminal wall of a vessel from the *node* of *G. paniculatum*, Benth., showing a double row of round perforations. × 250.

Fig. 38. Terminal wall of a vessel from the internode of a young stem of *G. Gnemon*, L., showing a transitional stage between the double and the single row of perforations. × 250.

Fig. 39. Terminal wall of a vessel from the region between the node and internode, showing the irregular mode of fusion of the perforations in *G. paniculatum*, Benth. × 165.

Fig. 40. Terminal wall of a vessel close to the node, showing a variety in the arrangement of the perforations in *G. paniculatum*, Benth. × 165.

Fig. 41. Terminal wall of a vessel from the wood of *Ephedra*, showing the double row of perforations, and three bordered pits at its extremity. × 250.

Fig. 42. Transverse section through a bundle of the leaf of *Ephedra*, showing two transfusion-elements at its edge (*tf*). *x*, xylem; *ph*, phloëm; *ms*, mesophyll. × 250.

Fig. 43. Terminal walls of three vessels occurring next to the protoxylem (*px*), from a twig of *Carpinus*, showing the mode of perforation in these first-formed elements. × 250.

Fig. 44. Terminal wall of a similar vessel isolated. × 250.

Fig. 45. Terminal wall of a tracheide from the wood of *Corylus*, showing a transitional stage between bordered pits and ladder-like perforations. × 250.

On Correlation in the Growth of Roots and Shoots[1].

BY

L. KNY.

EVERY multicellular plant-body—the cells of which are not loosely connected as are those of a colony, but, on the contrary, maintain a mutual interchange of material and present a diversity of complementary functions —constitutes a single and complete whole. In the lower plants this unity is not fully attained, for the independent life of each individual cell still asserts itself too strongly: here each cell possesses the capacity of performing all, or at any rate many, of the vital functions of the organism, and consequently one part of the plant can continue to live independently of another. But in plants, such as the Phanerogams, where the physiological division of labour reaches its highest pitch, this is not the case: to such plants the foregoing proposition is more especially applicable. Here root, stem, and leaf mutually presuppose each other. A root no longer in structural connexion with its corresponding shoot, clearly lacks all the conditions for prolonged existence; for it can no longer get rid of the materials which it absorbs from the soil, nor does it receive supplies of organic substance for its nutrition. Similarly, stem and leaf can only continue to function normally so long as the regular interchange of material between them and the other members of the plant with which they are in organic connexion is maintained.

Such considerations as these justify the question raised by

[1] Read before Section D of the British Association, Oxford, August 11, 1894.

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Voechting [1], 'whether there does not exist a relation of symmetry between root and bud such that when the development of the one is prevented, that of the other will not take place?'

Certain facts in horticultural experience, especially the culture of dwarf trees in pots [2], tend to support this view: but on the other hand, there are many facts which go to prove that the various members of the plant-body do not always develop at the same rate.

When the seed of a normal chlorophyll-containing Phanerogam germinates, the radicle usually escapes first from the testa, and attains a certain length before there is any external indication of the development of the plumule. In some cases the degree to which this early root-development takes place is very remarkable. Thus G. Haberlandt [3] states that the primary root of the Date-seedling may attain a length of as much as a metre before the first foliage-leaf unfolds. According to Volkens [4], a very great disproportion exists between the root and the shoot of Desert-plants, both as regards their length and the rate of their growth. Again, in certain epiphytic Aroids, in *Carludovica Plumieri*, and in *Clusia rosea*, the aërial roots which convey nourishment for the plant to a height of 100 feet and more above the ground, it is clear [5] that the development of the roots has taken place at the expense of that of the shoot. The same is still more markedly the case in some Orchids (*Angraecum globulosum* [6], *Aeranthes funalis* [7]), and in some Podostemaceae [8]

[1] Voechting, Ueber Organbildung im Pflanzenreiche, I, p. 51, 1878.

[2] Voechting, l. c., II, pp. 97–107, 1884. See also Reinke, 'Die Abhaengigkeit der Blattentwickelung von der Bewurzelung,' Ber. d. deutsch. bot. Gesellsch., II, p. 376, 1884.

[3] Eine botanische Tropenreise, p. 287, 1893.

[4] Die Flora der aegyptisch-arabischen Wüste, pp. 23–26, 1887.

[5] A. F. W. Schimper, 'Die epiphytische Vegetation Americas,' Bot. Mittheilungen aus den Tropen, II, p. 54, 1888.

[6] Pfitzer, Grundzüge einer Vergleichenden Morphologie der Orchideen, cited by Goebel, Vergleichende Entwickelungsgeschichte der Pflanzenorgane, p. 126, 1884.

[7] Schimper, l. c., p. 48.

[8] Warming, in Engler's Bot. Jahrbücher, IV, pp. 220–2, 1883.

(*Hydrobryum olivaceum*, and species of *Dicraea*), where the roots are developed as the most important organs for the assimilation of carbon dioxide, whilst the leaves are more or less rudimentary, and the stem is reduced to little more than the peduncle of the inflorescence.

A contrast to the foregoing examples is afforded by those plants in which the stem and its branches are more or less independent of the root. Every plant-collector knows that in many species of *Sedum*, the leafy branches continue to grow for a time after the plants have been placed in the herbarium, unless they have been previously killed by immersion in hot water. Volkens[1] states that a rootless plant of *Mesembryanthemum crystallinum* continued to live for several weeks without any supply of water, and even produced flowers: and the experience of growers of Cactaceae proves that rootless pieces of stem of *Opuntia* and other genera will throw out new branches in dry air. The cut-off rootless ends of shoots of *Elodea canadensis* may often be observed growing in the water, without any immediate root-formation having taken place. The extreme in this direction is reached by those plants which are rootless in the adult stage, the functions of the root being discharged by other organs: such plants are, among Vascular Cryptogams, *Salvinia natans*, *Psilotum triquetrum*, and possibly some Hymenophyllaceae such as *Trichomanes emarginatum* and the species of the genus *Hemiphlebium*[2]; among Phanerogams, *Lemna arrhiza*, *Corallorhiza innata*, *Epipogium Gmelini*, *Tillandsia usnoides*[3], species of *Ceratophyllum*, all the species of *Utricularia* examined by Goebel[4], and *Aldrovanda vesiculosa*.

But these cases in which the morphological equilibrium between root and shoot is so considerably disturbed, are exceptions. The normal cases are those of chlorophyll-

[1] Loc. cit., p. 53.
[2] Prantl, Unters. zur Morphol. der Gefaesskryptogamen, I, pp. 29-31, 1875.
[3] Schimper, l. c., II, p. 68.
[4] Goebel, Flora, p. 291, 1889.

containing terrestrial plants in which the sub-aërial and the subterranean parts are developed in due proportion: and it is with reference to these latter that the following interesting questions suggest themselves:—

1. Does there exist between the roots and the shoots of a seedling such a correlation that the removal of the roots inhibits the development of the shoots, and *vice versa*? Or is it not rather the case that the removal of the one part induces a more vigorous development of the other on account of the larger quantity of plastic material now available? Or does the growth of the one part stand in no relation whatever to that of the other, in the developing seedling?

2. What is the limit to which the development of the shoots of seedlings will proceed after continued removal of the roots, and the development of the roots after continued removal of the shoots?

3. Are the phenomena of this kind which can be observed on isolated parts of adult plants (cuttings, tubers, &c.) different from those which are manifested by seedlings?

A. Experiments with Seedlings.

It is obvious that some seeds are more suitable than others for experiments of this kind: the seed must contain either abundant endosperm or fleshy cotyledons, so that the developing seedlings may have an adequate supply of organic nourishment available.

If the experiments are to be carried out with complete exactitude, the seedlings must be grown, not in water, but in air saturated with moisture; otherwise the uninjured seedlings will not be comparable with those whose roots have been removed, for the root is above all things organized for the absorption of water in the liquid form. Unfortunately there are difficulties in the way of practically carrying out this condition. The seeds of some species, such as *Helianthus annuus*, grow but little when kept on dry glass plates over water in bell-jars, and their roots soon show symptoms of drying up. *Zea Mays* proved itself to be the most suitable

seed for the experiment, as its moistened endosperm acts to a considerable extent as a water-reservoir for the seedling: but even in *Zea Mays* the seedlings grew much better when the soaked seeds were laid on damp plates of burnt clay. In this case, though the most strongly developed root hung freely down in the damp air, it was impossible to prevent the contact of the finer roots with the damp plate; but since, in consequence of their positive geotropism, they nearly all had their apices pressing against the surface of the plate, the portions bearing the root-hairs were for the most part not in contact with the plate, but arched in the air.

In the case of *Vicia Faba*, I found it advantageous to place each seedling with one cotyledon resting on some moist sand in a very small porcelain dish, so that the tap-root grew over the edge of the dish into the damp air, the sand being moistened every day or two by means of a pipette: the seedling was thus adequately supplied with water, although it was impossible for the tap-root to absorb any in the liquid form.

The details of the arrangement of the experiment were as follows: A number (a multiple of 3) of tubulated bell-jars of equal size were used: each bell-jar had a clear width of 15·5 cm., and a clear height of 16 cm. to the tubulure, and it stood in a porcelain saucer 20·5 cm. in diameter containing enough water to cover the rim of the bell-jar. Inside each bell-jar was an inverted flower-pot, of about two-thirds the height of the bell-jar, and on this was placed either a glass dish or a circular porous earthenware plate, accordingly as a dry or a damp substratum was required. The inner surface of the bell-jar was sprinkled with drops of water. In order to provide a slight supply of air, the tubulure of the bell-jar was covered by an inverted glass beaker with a lip.

The bell-jars were arranged in three series. In those of the first series were placed the normal seedlings, either altogether uninjured (*Zea Mays*) or with one cotyledon removed (*Vicia Faba*): in both cases plumule and radicle were intact and could develop unhindered. In those of the second series were placed seedlings in which the plumule had

been removed by means either of a pointed scalpel or of a specially adapted pair of scissors. In those of the third series were placed seedlings in which the roots had been removed, the plumule being left. In other respects the seedlings in the three series were quite similar. It was, however, found necessary, in the case of *Vicia Faba*, to remove one of the cotyledons in the seedlings of all three series; for otherwise it would have been impossible to remove the plumule with a clean cut. Previously to the commencement of the experiments, the seeds to be used were kept for some time, until the protrusion of the radicle, in shallow dishes either with water (*Vicia Faba*) or on very pure wet sand, and were carefully selected so that the seedlings of all three series might be equally vigorous and equally developed in the same time. The germinating seeds were so placed on the glass dish or earthenware plate under the bell-jar, that the tap-root (*Vicia Faba*) or the most vigorous root (*Zea Mays*) could only grow over the edge into the moist air.

In the more rapidly growing *Zea Mays*, the seedlings were inspected every day; in *Vicia Faba* they were inspected every day or every two days as circumstances required. In order to neutralize the injurious effect of a short exposure to dry air, the seedlings on inspection were at once placed, after the removal of the bell-jar, in a dish of water, and then, in the case of the seedlings of Series 2 and 3, any newly formed adventitious shoots in the former, and any newly formed adventitious roots in the latter, were carefully removed. In order to maintain, as far as possible, uniformity of conditions, the seedlings of Series 1 were also placed, at each inspection, for a time in a dish of water. In all cases the water was, as far as possible, shaken off the seedlings before they were replaced under their respective bell-jars.

Each experiment was terminated when the roots had nearly reached the water in the saucer under the bell-jar. By this time the shoots had grown so that they had almost or quite reached the curved roof of the bell-jar.

The method of weighing was adopted for the purpose of

estimating the results. Measurement would, especially as regards the roots, have given quite unreliable data, for it might well happen that, with a short main root, the number and bulk of the lateral roots should be relatively large. Moreover, the determination of the gross weight of the harvested roots and shoots would not be a sufficiently accurate method, since the loss of water by evaporation during the process of weighing would not be equal in all cases. Hence, in several experiments, the *dry* weight of the roots and shoots was determined after several hours' exposure to a temperature of 100°–110° C.

In addition to the results of the experiments carried out in the manner already described, I also append those of some others in which the seedlings were not laid on glass or on earthenware, but on a thin layer of moistened silver-sand spread on glass. In these, only Series 1 and 2 are strictly comparable; for in Series 3 the seedlings were obviously at a disadvantage, as compared with those of Series 1, in that they were deprived of the means of absorbing the available liquid water.

At the conclusion of all the experiments it was ascertained that starch was still abundantly present in the endosperm or the cotyledons, as the case might be, so that even the most fully developed seedling was still provided with plastic material for further growth.

1. Cultures of *Zea Mays* on moist plates of burnt clay.

No. of Expt.	Commt. of Expt.	End of Expt.	No. of Bell-jars in each Series.	No. of Seedlings in each Series.	Series.	Length of the longest Root.	Length of the longest Shoot.	Total Gross Weight of Roots.	Total Gross Weight of Shoots.	Total Dry Weight of Roots.	Total Dry Weight of Shoots.
	1894 June				1	93 mm.	40·5 mm.	2·73 grm.	1·75 grm.	0·251 grm.	0·176 grm.
1	19	23	2	24	2	96 mm.		2·55 grm.		0·269 grm.	
					3		38·5 mm.		1·72 grm.		0·209 grm.
					1	110·5 mm.	41 mm.	2·810 grm.	2·360 grm.	0·344 grm.	0·239 grm.
2	26	29	2	24	2	122 mm.		2·932 grm.		0·345 grm.	
					3		40·5 mm.		2·313 grm.		0·290 grm.
	July				1	98 mm.	55·5 mm.	3·230 grm.	3·851 grm.	0·369 grm.	0·372 grm.
3	2	5	3	36	2	105 mm.		3·472 grm.		0·424 grm.	
					3		35·5 mm.		2·635 grm.		0·342 grm.

2. Culture of *Zea Mays* on damp sand.

No. of Expt.	Commt. of Expt.	End of Expt.	No. of Bell-jars in each Series.	No. of Seedlings in each Series.	Series.	Length of the longest Root.	Length of the longest Shoot.	Total Gross Weight of Roots.	Total Gross Weight of Shoots.
1	June, 1894 2	9	2	16	1	154 mm.	95 mm.	4·4 grm.	3·75 grm.
					2	123 mm.		5·35 grm.	
					3		34·5 mm.		1·35 grm.

In this experiment the determination of the dry weight was omitted, since it was found impossible to free the roots from adhering sand without injury.

3. Cultures of *Vicia Faba*. The one cotyledon was resting on damp sand in a small dish.

No. of Expt.	Commt. of Expt.	End of Expt.	No. of Bell-jars in each Series.	No. of Seedlings in each Series.	Series.	Length of the longest Root.	Length of the longest Shoot.	Total Gross Weight of the Roots.	Total Gross Weight of the Shoots.	Total Dry Weight of the Roots.	Total Dry Weight of the Shoots.
	June, 1894				1	77 mm.	34 mm.	3·87 grm.	2·4 grm.	0·498 grm.	0·322 grm.
1	13	21	3	15	2	49·5 mm.		4·05 grm.		0·475 grm.	
					3		37·5 mm.		2·95 grm.		0·399 grm.
	July, 1894				1	82 mm.	36 mm.	6·635 grm.	3·360 grm.	0·640 grm.	0·368 grm.
2	13	21	3	15	2	72·5 mm.		5·985 grm.		0·589 grm.	
					3		52·5 mm.		4·091 grm.		0·429 grm.
					1	79 mm.	53·5 mm.	4·982 grm.	3·893 grm.	0·435 grm.	0·356 grm.
3	23	28	3	15	2	77 mm.		4·952 grm.		0·424 grm.	
					3		49·5 mm.		4·652 grm.		0·408 grm.

4. Cultures of *Vicia Faba*, carried further than those in 3. The seedlings were kept throughout on a layer of moist pure quartz-sand, on an inverted glass saucer at about one-third the height of the bell-jar. At the close of the experiment the roots had branched abundantly in the water in which the bell-jar was standing.

No. of Expt.	Comm. of Expt.	End of Expt.	No. of Bell-jars in each Series.	No. of Seedlings in each Series.	Series.	Length of the longest Root.	Length of the longest Shoot.	Total Gross Weight of Roots.	Total Gross Weight of Shoots.
1	1894 March 21	April 3	3	15	1			19·3 grm.	11·9 grm.
					2			22·4 grm.	
					3				4·5 grm.
2	April 4	April 18	3	15	1	206 mm.	130 mm.	23·55 grm.	10·9 grm.
					2	220·5 mm.		21·8 grm.	
					3		44·5 mm.		2·75 grm.
3	April 23	May 9	3	15	1	184 mm.	109 mm.	18·75 grm.	8·5 grm.
					2	157 mm.		25·0 grm.	
					3		36 mm.		2·9 grm.

The determination of the dry weight was omitted for the same reason as in Culture 2.

In these three last experiments it is readily observable that the primary shoots of Series 3 gained an advantage, as compared with those of Series 1, in the first few days; whereas at the close of the experiment the shoots of Series 1 had the advantage as compared with those of Series 3.

B. Experiments with Cuttings.

As these experiments require a great deal of space and take a long time, I have only been able to make two with cuttings of *Salix acuminata*, Sm., and *Salix purpurea*, L. A third experiment made with cuttings of *Hedera Helix* gave no satisfactory results, inasmuch as it was begun at a time when the winter-buds had already begun to sprout and when, consequently, the store of reserve material was already partly exhausted.

If the experiments with cuttings are to be carried on for several months, the method employed with the seedlings cannot be adopted: for cuttings suspended by wires in a saturated atmosphere soon become covered with mould. I was obliged, therefore, to adopt a mode of experimentation which allows the intact cuttings to be compared only with those whose sprouting green shoots are removed. Rooted cuttings are not strictly comparable with those whose developing roots are repeatedly removed, as the former absorb a greater amount of liquid water.

In view of these conditions, the following arrangements were made. Nine glass cylinders, of about 16 cm. clear diameter and of about 4·5 litres content, were taken, each being loosely closed by a wooden cover. In each cover a central and six peripheral holes were made, seven holes in all, at equal distances, each hole having a diameter of about 20 mm.

On Feb. 7, 1894, the nine cylinders were filled with water nearly to the brim, and in each hole a cutting of about 27 cm. in length was fixed by means of wadding. The diameter of the cuttings varied from 9 to 15·5 mm. The length of the cuttings below and above the wooden cover was about the same.

The nine cylinders were arranged in three series of three each; and care was taken that each series included cuttings of large, medium, and small diameter, in equal proportion. With this object in view, the cuttings were carefully assorted in a large vessel full of water, before the commencement of the experiment. All the nine cylinders were placed in the cold house of my Botanical Laboratory.

At intervals of from two to seven days, according to the prevailing conditions of temperature, the cylinders were inspected, and the water which they contained was occasionally changed during the course of the experiment.

In Series 1, the cuttings remained uninjured throughout the whole course of the experiment. In Series 2, the developing buds, and in Series 3 the developing roots, were carefully removed. For this purpose the covers were lifted, and the operation was rapidly performed with scissors. In order that the conditions in Series 1 might be identical with those of Series 2 and 3, the cuttings of Series 1 were lifted out of the water, and the roots exposed to the air, for the same length of time.

The experiment was concluded on April 11, that is after two months and four days. An examination of the wood for the presence of starch showed that it was only completely absent from the stoutest cuttings of Series 1 and 3, whilst it was still to be detected in the wood-parenchyma and in the medullary rays of all the others.

It was already noticeable on March 10, that the roots of those cuttings whose developing shoots had been removed (i. e. Series 2) were, on the whole, shorter than those of the uninjured cuttings (Series 1). By March 28, this difference had become still more apparent. In Series 1, the roots were more numerous and longer, and bore numerous lateral roots attaining a maximum length of 2·5 cm. In Series 2, the roots were shorter and less numerous; some of them bore no lateral roots, whilst others bore lateral roots only a few mm. long. At the close of the experiment on April 11, tertiary root-branches were developed to some extent in Series 1,

whereas this was not the case in Series 2, where some of the main roots were still altogether unbranched.

By March 10, there was no perceptible difference between Series 1 and 3 as regards the development of shoots. On March 28 there was a distinct difference in this respect, in favour of Series 1, although the difference was not so marked as that between the roots of Series 1 and 2. In Series 1, the shoots had attained a maximum length of 232 mm.; whereas in Series 3 the maximum length was only 185 mm. At the close of the experiment on April 11, the difference in length was still more striking.

The numerical results were as follows:—

Series 1.—Cuttings uninjured throughout the experiment:

Length of the longest root . . .	279 mm.
Total gross weight of the roots . .	23·95 grm.
Total dry weight of the roots . .	2·197 grm.
Length of the longest shoot . . .	376 mm.
Total gross weight of the shoots . .	121·1 grm.
Total dry weight of the shoots . .	21·5 grm.

Series 2.—Cuttings from which the shoots were repeatedly removed:

Length of the longest root	220·5 mm.
Total gross weight of the roots . .	4·55 grm.
Total dry weight of the roots . . .	0·337 grm.

Series 3.—Cuttings from which the roots were repeatedly removed:

Length of the longest shoot . . .	216 mm.
Total gross weight of the shoots . .	65·35 grm.
Total dry weight of the shoots . .	14·7 grm.

I do not give the details of an experiment carried out a year earlier with cuttings of *Salix*, because in that case the cuttings were not so carefully assorted according to their diameter as to ensure uniformity in the three series. It will suffice to say that the results agreed in all important points with those given above.

The general conclusion to be drawn from my experiments is that, in the seedlings of the species employed, the growth of the roots and that of the shoots proceed with a high degree of independence.

In *Zea Mays*, the dry weight of the roots at the end of the experiments was, on the average, very much the same whether the shoots had been repeatedly removed or whether they had been allowed to remain. The same is true of the shoots with reference to the presence or absence of the roots. In *Vicia Faba*, the primary shoots of those plants whose roots were removed could be readily observed to have at first developed more vigorously than the primary shoots of those plants whose roots were not removed; whereas at the close of the experiment the contrary was the case, as is clearly proved by the very considerable difference between the gross weights (4. p. 275). The roots of those seedlings of *Vicia Faba*, from which the shoots were removed, showed, in the three last experiments related above, no diminution: on the contrary, the average weight of the roots formed is rather greater than in the case of the intact plants (Series 1).

The remarkable independence of development of the roots was apparent in other experiments made with the object of ascertaining, in seedlings of *Zea Mays*, *Phaseolus multiflorus*, and *Vicia Faba*, to what length the roots would grow in water when the primary shoot and all subsequently developing shoots were removed. In *Zea Mays*[1] the roots attained a maximum length of 630 mm.; in *Phaseolus multiflorus*, a length of 661 mm.; in *Vicia Faba*, a length of 718 mm.

An experiment was also made with seedlings of *Vicia Faba*, to ascertain the effect of removal of the roots; the seedlings were placed in moist garden soil, and were kept in the cold greenhouse. Although this experiment involved considerable disturbance of the seedlings in consequence of

[1] In *Zea Mays*, Van Tieghem (Ann. d. Sci. Nat., Bot., Sér. V, t. 17, 1873, p. 217) has already observed that in mutilated seedlings, which retained only the scutellum, there was a considerable development of roots, without any regeneration of the plumule or leaves having taken place. No measurements are given.

repeated digging up and replanting, no flagging of the shoots took place until they had attained a maximum length of 465 mm.

In the cuttings of *Salix*, the effects upon the shoots of removing the roots, and upon the roots of removing the shoots, made themselves apparent relatively early. The first thing to be noticed was a diminution in the development of the roots of those cuttings whose shoots had been removed: and then, somewhat later, the diminished development of the shoots of those cuttings whose roots had been removed became apparent. By analogy with the seedlings, just the opposite result might have been anticipated; for in the seedlings it was the roots which asserted their independence of the shoots the longer.

It remains to determine by more extended investigation to what extent the principles of correlation manifested in the growth of these few species are of general applicability.

The Periodic Reduction of the number of the Chromosomes in the Life-History of Living Organisms[1].

BY

EDUARD STRASBURGER.

THE simplest organisms with which we are acquainted reproduce themselves in only an asexual manner. It would appear that it is only in the lowest organisms that the absence of sexual differentiation is possible, and that this differentiation necessarily accompanies a certain definite degree of organization: it is, in fact, as if this differentiation must manifest itself at a certain stage of phylogenetic evolution in virtue of certain properties possessed by organized matter as such. It is true that many highly organized plants are asexual, but comparative investigation proves that this is due to a gradual loss of sexual differentiation, as in the great group of the Fungi, and doubtless also in the apogamous Ferns.

It appears that the sexual act has always given a powerful impulse to phylogenetic evolution; and that, on the other hand, all advance in development was in abeyance so long as sexual differentiation had not been obtained. From the phylogenetic standpoint, we must assume that all sexually differentiated organisms are descended from asexual organisms. The process of this descent is clearly illustrated in

[1] Translation of a paper communicated to Section D of the British Association, Oxford meeting, August, 1894.

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certain Chlorophyceae, in which the sexual act consists in the coalescence of swarming gametes. These gametes are obviously derived from asexual swarm-spores, which they quite resemble except in that they are smaller and often have fewer cilia.

The sexually differentiated plants manifest certain differences in their ontogeny, from which it is possible to infer what was the course along which the phylogenetic differentiation proceeded after sexual differentiation had taken place. The simplest case is that in which the product of fertilization gives rise to an individual similar to those which gave rise to the product of fertilization; and which closes its own life-history with the development either of sexual organs or of asexual organs homologous with them. This occurs in many Chlorophyceae, where, from the zygospore (the product of the coal-escence of similar gametes) or the oospore (the product of the coalescence of dissimilar spermatozoids and ova), a generation is developed which resembles the preceding and gives rise either to swarm-spores or to sexual cells homologous with them. Generally, any one sexual generation follows after a number of asexual generations, the relation being, however, dependent on external conditions, so that, as Klebs has shown, the development of a sexual or an asexual generation can be determined by the observer. In such cases there is a homogeneous sequence of generations which does not include any other kind of sequence or alternation beyond the development either of asexual reproductive organs or of sexual organs homologous with them. The asexual reproductive organs are especially concerned with the rapid multiplication of the individuals under favourable external conditions; whilst sexual reproduction is of importance in maintaining the existence of the species under circumstances which are unfavourable to the vegetative existence of the individual. At the same time, sexual reproduction ensures certain advantages which arise from the coalescence of distinct sexual cells.

In proportion as the asexual mode of reproduction was replaced by the sexual, the numerical conditions of multipli-

cation were maintained either by the development of a number of oospores, as in certain Fucaceae; or, in addition to the sexual organs, altogether new organs were developed to ensure rapid and vigorous development of new individuals in an asexual manner. This took place in various ways. Either asexual reproductive organs were intercalated in the life-history of the original generation, or an altogether new asexual generation was developed from the product of the sexual act. The independent individualization of these different stages of development of the sexual generation into special organs for vegetative multiplication, or into distinct bionts, was carried out to the highest degree in the Fungi and led to the evolution of the many different reproductive forms occurring, for instance, among the Ascomycetes. These arrangements for asexual reproduction were so efficient in the Fungi that the result was the disappearance of the sexual organs and of sexual reproduction. In the Mosses, on the one hand, and in the Vascular Cryptogams and Phanerogams, on the other, there sprang an altogether new generation from the product of the sexual act, the function of which is to reproduce asexually a large number of individuals. The degree of development attained by this generation differed accordingly as its activity was entirely limited to reproduction, or included also nutritive functions. In the Muscineae, this generation is restricted to the asexual multiplication of the individual, and hence it is. in these plants, the sexual generation in which the thallus has attained cormophytic differentiation into stem and leaf. In the Vascular Cryptogams, the centre of gravity of phylogenetic evolution is transferred to the asexual generation springing from the product of the sexual act: this is the generation which, in these plants, attained and advanced in cormophytic differentiation. In proportion as this evolution took place, the nutritive apparatus of the sexual generation became of less importance; and it became altogether superfluous from the moment when the asexual generation began to provide its spores with the material necessary for the development of the sexual generation. In accordance with

the general law which determines the phylogenetic disappearance of organs which have become useless, the vegetative parts of the sexual generation became more and more reduced, until little was left but the reproductive organs themselves: hence the progressive reduction in the prothallium from the Ferns up to the Phanerogams. This reduction culminated in the complete loss of independent existence by the sexual generation, because it had ceased to be able to nourish itself independently, and its becoming enclosed by the asexual generation. In consequence of this enclosure of the sexual in the asexual generation, the advantageous rapid multiplication of individuals which the latter originally effected was lost: in order to compensate for this loss, a large number of seeds were produced in the Phanerogams in place of the numerous spores of the Cryptogams; that is, multiplication is effected now by the products of fertilization instead of by asexual spores.

Alternation of generations is absolutely necessary only in those groups of plants in which the fertilized ovum gives rise to the asexual generation, and the asexual spore to the sexual generation. In all such plants the asexual generation is the product of a sexual act. It is important to draw attention to this fact, since it forms the basis of the views which will be subsequently expounded.

From what has been stated in the foregoing paragraphs, it it is clear that throughout the Plant-Kingdom (as far as sexuality is present), sexual differentiation was preceded by asexuality. On the other hand, it must be clearly apprehended that in all those divisions of the Plant-Kingdom in which a true alternation of generations obtains—that is, a necessary alternation of a sexual with an asexual generation—as in the Muscineae, Vascular Cryptogams, and Phanerogams, the sexual generation is to be regarded as the older and as having arisen from an asexual form. Similarly, comparative investigation teaches that the second generation, developed from the product of a sexual act, must be phylogenetically the younger. The gradual development of this generation from

the sexual product of the first generation can be actually traced step by step phylogenetically. The first indication of this development is apparently to be found in the Algae: at least the life-history of *Oedogonium*, *Coleochaete*, and the Florideae, may be interpreted in this sense. In *Oedogonium*, four swarm-spores are formed from the fertilized ovum; whilst in *Coleochaete* a small multicellular body is developed, from the cells of which swarm-spores are formed: in both cases the swarm-spore gives rise to the first generation. In the Florideae the cystocarp is developed from the fertilized ovum, and the spores of the cystocarp give rise to individuals of the first generation. The Muscineae and the Pteridophyta can readily be traced to the Chlorophyceae: in the Muscineae the fertilized ovum gradually developed into a sporogonium, and, in the Pteridophyta, into a sporangium-bearing cormophytic plant.

In the consideration of the alternation of generations obtaining in all the higher plants, importance is chiefly attached to the sexuality and asexuality of the two alternating generations respectively. But, as a matter of fact, it would be more accurate to lay emphasis on the mode of origin of the two generations: from this point of view the sexual generation would be characterized as the gamogenic or sexually-developed generation.

Our insight into the nature of the process of fertilization was very materially promoted by the discovery, made by Edouard van Beneden[1], that the number of the chromosomes is the same in both the conjugating nuclei. Further investigations established the fact, for both animals and plants, that a reduction to one-half of the number of the chromosomes in the generative nuclei precedes the sexual act, and that, in consequence of the coalescence of the male and female nuclei, the nucleus of the fertilized ovum possesses the number of chromosomes characteristic of a vegetative cell.

Basing myself on the observations which had been made

[1] Rech. sur la maturation de l'œuf, la fécondation, et la division cellulaire, Arch. d. Biol. IV, 1883, p. 403

on plants, I have[1], from the very first, maintained the view that the reduction in the number of chromosomes in the generative nuclei is not effected by the extrusion of some of them during the ripening of the sexual cells, but that, on the contrary, the development of the sexual cells depends on indirect nuclear division with longitudinal splitting of the chromosomes; and these views are also held by many observers as regards animals, and by Guignard[2] as regards plants. Guignard and I[3] established the fact that the number of chromosomes characteristic of the generative nuclei of Angiosperms, is determined, in the one case, in the mother-cells of the pollen, and, in the other case, in the mother-cells of the embryo-sacs. The investigations of the zoologists have also shown that this determination is effected in the mother-cells of the ova and of the spermatozoa of animals, by two successive cell-divisions, which give rise, in the one case, to four spermatozoa, and, in the other, to the ovum and to three so-called polar bodies. Guignard[4] in particular has endeavoured to follow with absolute accuracy all the processes affecting the reduction of the number of the chromosomes in the pollen-sacs and ovules of Lilies. The reduction takes place directly, both in the mother-cells of the pollen and in the mother-cell of the embryo-sac, and in such a manner that the reduced number of chromosomes is at once apparent in the prophase-stage. In all the preceding nuclear divisions, both in the pollen-sac and in the ovule, twenty-four chromosomes are, almost uniformly, visible: the framework of the resting-nucleus of the pollen-mother-cell and of the embryo-

[1] Neue Unters. üb. den Befruchtungsvorgang, 1884, pp. 16, 82: Ueb. Kern- und Zell-Theilung, 1888, p. 232.

[2] Études sur les phenomènes morphol. de la fécondation, Bull. de la Soc. Bot. de France, XXXVI, 1889, p. 106, &c.

[3] Strasburger, Ueb. Kern- und Zell-Theilung, 1888, pp. 51 and 240 ff.: Guignard, loc. cit., p. 105 ff.; and Nouvelles Études sur la Fécondation, Ann. Sci. Nat., Bot., Sér. 7, t. XIV, 1891, p. 246 ff.: compare also, Overton, Beitr. z. Kenntniss der Geschlechtsproducte des *Lilium Martagon*, Festschrift für Kölliker und Naegeli, 1891.

[4] Nouvelles Études, pp. 173, 182.

sac-mother-cell is therefore constructed of twenty-four chromosomes: yet in the next prophase it nevertheless uniformly gives rise to only twelve chromosomes. In connexion with this reduction the nucleus does not undergo any diminution either in size or in mass: on the contrary, those nuclei in which the reduction in the number of the chromosomes takes place are remarkable for their size and for the abundance of chromatin which they contain. I contrasted, also in *Lilium*, the cell which gives rise to the embryo-sac with the mother-cells of the pollen; but it must be borne in mind that in *Lilium*, as in *Tulipa* and *Fritillaria*, this cell develops directly into the embryo-sac without undergoing that division which, in other cases, distinguishes the cell as a mother-cell. Hence these observations left open the possibility that the reduction in the number of the nuclear chromosomes might take place in the young embryo-sac and not in the mother-cell of the embryo-sac. I have, however, succeeded [1] in determining that the reduction of the chromosomes to eight or twelve in the embryo-sac-mother-cells of the ovules of *Allium* and *Helleborus* respectively, takes place before they have undergone their characteristic divisions. Hence the cell of *Lilium*, in which the reduction in the number of the chromosomes takes place, must undoubtedly be regarded as an embryo-sac-mother-cell, the course of development being abbreviated. The successive divisions of this cell, in those cases in which they actually take place, give rise, in addition to the embryo-sac, only to reduced cells which are immediately compressed and absorbed. The idea suggested by Bütschli [2] that the polar bodies of animals may be reduced ova was definitively established by O. Hertwig's [3] comparative investigation of the development of the ova and spermatozoa of the Nematodes. The mother-cell of the egg in animals gives rise, by two successive divi-

[1] Ueb. Kern- und Zell-Theilung, p. 243.

[2] Gedanken üb. die Morphol. Bedeutung der sogenannten Richtungskorper, Biol. Centralbl., IV, p. 5, 1884.

[3] Archiv fur mikr. Anat., Bd. 36, 1890.

sions, to four cells, and so does the sperm-mother-cell[1]. But whilst the products of division are, in the latter case, all similar, each developing into a spermatozoon, in the former only one develops into a fertile ovum, the other three are polar bodies, that is, they are merely reduced ova. Whilst in plants a reduction in the number of the chromosomes takes place in an unmistakable manner in the nuclei of the mother-cells of the pollen and of the embryo-sacs, it appears, on the contrary, as if in the Nematodes a doubling of the number of chromosomes took place, in the first instance, in the mother-cells of the ova and spermatozoa. But this increase in the number of the chromosomes is only apparent, for it is dependent upon the fact that, in this case, the chromosomes for the two following divisions are provided by longitudinal splitting before they have begun. The phenomenon is merely one of abbreviation: it is nothing more than the compression into one of the longitudinal splittings of the chromosomes attending two successive divisions. The reduction of the number of the chromosomes to one-half thus only becomes apparent in the spermatozoa, the ovum, and the polar bodies, although, as a matter of fact, it takes place in the mother-cells.

But what is the significance of this reduction in number of the chromosomes in the sexual cells, and of the equality of their number in the male and female cells? The physiological utility of the arrangement readily suggests itself: for were it not so, the number of chromosomes in the nuclei of each generation would be twice as great as in the preceding; and again, by this means each parent is represented in the offspring by an equal number of chromosomes, and thus equally transmits its hereditary characters. The morphological cause of the reduction in number of the chromosomes and of their equality in number in the sexual cells is, in my opinion, phylogenetic. I look upon these facts as indicating

[1] The literature relating to this subject is cited in my work 'Schwärmsporen, Gameten, pflanzliche Spermatozoiden, das Wesen der Befruchtung,' p. 151.

a return to the original generation from which, after it had attained sexual differentiation, offspring was developed having a double number of chromosomes. Thus the reduction by one-half of the number of the chromosomes in the sexual cells is not the outcome of a gradually evolved process of reduction, but rather it is the reappearance of the primitive number of chromosomes as it existed in the nuclei of the generation in which sexual differentiation first took place. Viewed from this standpoint, many facts become more readily intelligible: for instance, the immediate and sudden occurrence of the reduction, the developmental stage at which it takes place, and the varying length of the interval which separates it from the sexual act.

The number of chromosomes determined in the mother-cells of the pollen of the Angiosperms persists up to the formation of the spermatic nucleus in the pollen-tube. Four divisions are involved in the development of this nucleus: two divisions take place in the mother-cell resulting in the formation of four pollen-grains; then there is the division in the pollen-grain by which the generative and the vegetative cells are respectively formed; and, finally, there is the fourth division, the division into two of the generative cell in the pollen-tube. The number of chromosomes determined in the mother-cell of the embryo-sac persists through a series of divisions, the number of which varies with the species of plant, until it attains functional importance in the ovum. As a rule, the mother-cell of the embryo-sac divides twice, and the lowest of the resulting daughter-cells develops into the embryo-sac. In the embryo-sac three divisions succeed each other before the nucleus of the ovum is formed. In this case five divisions, and not four as in the development of the spermatic nucleus, intervene between the reduction and determination of the number of the chromosomes, on the one hand, and the constitution of the sexually-functional nucleus on the other. That the number of these intervening divisions is not of primary importance, is proved by the fact that the number is not always the same: thus, in *Lilium* and *Tulipa* there

are but three; in *Ornithogalum*, *Commelyna*, and species of *Agraphis*, there are four; in yet other cases the number is greater than five, as in *Rosa livida*[1], in which case, it should be mentioned, the moment at which the reduction takes place has yet to be ascertained and thus also the identity of the cell established which functions as the mother-cell of the embryo-sac. In view of these facts, it is not surprising that in *Scilla*[2] and *Ornithogalum* the division of the spermatic nucleus in the pollen-tube may be repeated, so that often four spermatic nuclei are formed instead of two. The attempts which have been made to establish homologies between the individual successive divisions which precede the formation of the nucleus of the ovum, on the one hand, and those which precede the formation of the spermatic nucleus, on the other, are thus shown to be futile: and equally barren is the attempt to establish, on physiological grounds, the necessity for a certain definite number of nuclear divisions, based on the assumption that it is by these successive divisions that the male and female nuclei are brought to the same bulk. The whole process appears in an altogether new light when considered from the point of view that the reduced number of chromosomes in the mother-cells in question is the expression of the original ancestral number of chromosomes existing before the sexually-produced generation had been evolved.

The reduction of the number of the chromosomes in the pollen-mother-cells of Angiosperms, which has been adduced as an example, is therefore not to be regarded as a preparation for the sexual act; it really marks the beginning of the new generation which comes into existence with the primitive number of chromosomes. This primitive generation has, however, undergone great limitation before it attained the reduced ontogeny which it now exhibits in the Angiosperms. In the first place it developed sexual dimorphism, so that it

[1] Strasburger, Angiospermen und Gymnospermen, 1879, p. 14, Taf. IV.

[2] Strasburger, Neue Untersuchungen üb. den Befruchtungsvorgang bei den Phanerogamen, 1884, p. 17.

was represented by two parallel developmental series, a male and a female. The phylogenetic course along which this reduction, as also the development of the dimorphism, proceeded, can be traced backwards.

Overton was the first to point out that, in the Gymnosperms also, the nuclei of the mother-cells of the pollen and of the embryo-sacs contain only half the number of chromosomes as compared with those of the plant developed from the ovum. He had already been rightly led by his researches on *Lilium* to raise the question[1] 'whether the reduction may not take place, in the Vascular Cryptogams and the Mosses, in those cells which are the morphological equivalents of the mother-cells of the pollen and of the embryo-sacs of the Angiosperms; in other words, whether the reduction does not take place in the mother-cells of the spores, that is, at the point of alternation of the generations.' In the mother-cells of the pollen of *Ceratozamia*, Guignard[2] counted eight chromosomes, and found that this number persisted through the subsequent divisions. Overton[3] found the same number of chromosomes in the developing endosperm in the embryo-sac. Guignard ascertained in *Ceratozamia*, and I in several Conifers[4], that all the nuclear divisions in the mother-cells of the pollen and in the pollen-grains themselves are accompanied by longitudinal splitting of the chromosomes. At the same time I drew attention to the uniformity in the number of the chromosomes in the pollen-grains and ova of the Conifers; and I also suggested the probability that in Gymnosperms also the number of the chromosomes is determined in the mother-cell of the embryo-sac[5]. This last point still remains to be proved, since

[1] Ueb. d. Reduction der Chromosomen in den Kernen der Pflanzen, Vierteljahrschr. d. naturforsch. Ges. in Zurich, XXXVIII, 1893; also previously in Ber. d. Schw. Bot. Ges., Heft III, 1893; Jahresber. d. Zuricher Bot. Ges., 1892-3, Sitzung von 21. Jan. 1892; and Annals of Botany, Vol. vii, No. XXV, March, 1893.

[2] Journal de Botanique, III, 1889, p. 232.

[3] Ueb. d. Reduction der Chromosomen, &c.

[4] Guignard, l. c.; Strasburger, Ueb. das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen, 1892, p. 34.

[5] Loc. cit., p. 35.

Henry H. Dixon[1] found it necessary, in his investigation carried on in the Botanical Institute of the University of Bonn, in the spring of 1893, to confine his attention to ascertaining the reduction by half of the number of chromosomes in the endosperm-tissue of *Pinus sylvestris*. It can, however, hardly be doubted that the reduction takes place in the mother-cell of the embryo-sac as it does in the mother-cell of the pollen. However, without going beyond what is actually ascertained, namely, that the reduction in the number of the chromosomes takes place in the developing endosperm long before the development of the archegonia has begun, there is sufficient evidence to prove that the number of successive divisions which the nuclei with the reduced number of chromosomes have to undergo is altogether different and without relation in the parallel male and female generations. For instance, in *Biota orientalis* only five nuclear divisions intervene between the mother-cell of the pollen and the development of the spermatic nuclei: the pollen-mother-cell divides twice; the pollen-grain divides once, forming the small generative and the larger vegetative cell; the generative cell divides once, and the anterior of the two cells divides in the pollen-tube, forming the two sexually functional cells[2]. With this are to be contrasted the very numerous nuclear divisions which must take place in the embryo-sac of this plant before tissue-formation begins, and, in addition, the number of nuclear divisions which intervene between commencing tissue-formation and the completed development of the archegonium. Dixon[3] counted, in *Pinus sylvestris*, only eight chromosomes in the nuclei of the young endosperm, as also in the ovum at the time of the formation of the canal-cell: on the other hand, I had counted twelve chromosomes in the pollen-grains of the same plant[4]. From renewed investigation made in the

[1] Fertilization of *Pinus sylvestris*, Annals of Botany, Vol. viii, No. XXIX, p. 21, 1894.

[2] Strasburger, Ueb. das Verhalten des Pollens, &c., bei den Gymnospermen, p. 19.

[3] Loc. cit., p. 29 ff.

[4] Ueb. das Verhalten des Pollens, &c., p. 34.

spring of this year, I come to the conclusion that both the pollen-mother-cells and the pollen-grains of *Pinus sylvestris* have only eight chromosomes. The counting of the chromosomes in this material is attended with great difficulty, and is somewhat uncertain, for the limits of the individual chromosomes are not clearly distinguishable, and moreover the chromatic segments in the chromosomes, when division is about to take place, are very distinct and often produce the impression of being independent chromosomes; hence it is easy to conclude that the chromosomes are more numerous than is really the case. The nuclei in the nucellus and in the integuments of the same species of *Pinus* were found by Dixon to contain sixteen chromosomes, and my older preparations clearly show that the dividing nuclei of the developing embryo in the lower end (the morphological apex) of the ovum of *Pinus sylvestris* have more than eight, probably sixteen, chromosomes. They agree, as regards the number of the chromosomes, with the figures of *Picea vulgaris* which I published in 1880[1].

Overton[2] has already drawn attention to the fact that the processes which go on in the spore-mother-cells of the Vascular Cryptogams and the Mosses so closely resemble those by which the reduction in the number of the chromosomes in the mother-cells of the pollen is effected, that their significance is probably the same in both. He found the actual determination of the number of the chromosomes to be attended with great difficulty in the Muscineae on account of the small size of the nuclei, and in the Pteridophyta on account of the large number of the chromosomes. As regards the Pteridophyta, although the number of the chromosomes is considerable in some of them, in others it is not greater than in the Phanerogams: for instance, in *Osmunda regalis* the chromosomes can be easily counted. I ascertained that there are twelve chromosomes in the spore-mother-cells of this plant. The differentiation of them in the

[1] Zellbildung und Zelltheilung, 3ᵉ Auflage, Taf. IV.

[2] Loc. cit.; p. 12 of the separate copy.

nucleus of the mother-cell as it leaves the resting stage takes place just as directly as in the pollen-mother-cells of the Phanerogams. It can be easily ascertained that this number persists through the two divisions which result in the formation of the four spores. On the other hand, the nuclei of the archesporial cells, previously to the differentiation of the spore-mother-cells, contain a larger number, probably twice as many or nearly so. This higher number persists, after the differentiation of the spore-mother-cells, in the external tissues of the sporangium. This is shown in the figures published by Dr. J. E. Humphrey[1], who investigated the nuclei of *Osmunda*, with regard to the behaviour of their centrosomes and nuclei, in my Botanical Laboratory last winter. Thus his Fig. 11 shows a mother-cell of *Osmunda regalis* undergoing the first division, and Fig. 12 a mother-cell undergoing the second division; whilst Fig. 10 also shows the division of a tapetal mother-cell. Prothallia, developing from spores sown in a culture-solution, showed twelve chromosomes in all stages, that is, the same number as in the spore-mother-cells. The search for nuclear divisions in developing prothallia requires a great deal of patience, for, so far as my experience goes, they do not take place at any particular time of the day, and hence they are only to be found in isolated cases. My attempts to arrest the nuclear divisions by exposure to low temperatures, or by absence of light, so that they might take place more frequently at appropriate times, led to no result: influences which had proved to be effectual in *Spirogyra* had in this case no effect. All that I could do was to examine a very large number of prothallia which had been fixed in alcohol at various times of the day, and had been stained. I was successful in carrying the counting of the chromosomes up to the commencing development of the antheridia and spermatozoids, and in all cases I found the number to be the same. It is unnecessary to say more than that the processes

[1] Bericht. d. deutsch. bot. Ges., 1894, Heft 5, Taf. VI: see Notes, this number.

which lead up, on the one hand, to the development of the numerous spermatozoids in the antheridium, and, on the other, to the development of the single ovum in the archegonium, differ both in the number and in the succession of the divisions. It is therefore impossible that the divisions taking place in the sexual organs can be of importance in the direction of ensuring equivalence of the sexual cells in preparation for the sexual act. Nor does a process of any kind whatsoever take place by which an equality in number of the chromosomes in the nuclei of the sexual cells is secured: this number is fixed once and for all in the mother-cells of the spores. It may therefore be concluded with regard to *Osmunda regalis*, and doubtless also with regard to all Ferns, that the nuclei of the sexual generation contain only half as many nuclei as do those of the asexual generation. Nor can there be any doubt that in the Ferns the sexual generation is the older: the second arose by progressive phylogenetic differentiation of the product of the sexual act after the first had become sexually differentiated, and hence its double number of chromosomes.

With regard to the Muscineae, the counting of the chromosomes has recently been undertaken by J. Bretland Farmer[1]. He found in *Pallavicinia decipiens*, a Liverwort from the mountains of Ceylon, that the dividing nuclei of the sexual generation (gametophyte) each contain four chromosomes. In the asexual generation (sporophyte) Farmer counted eight chromosomes in the nuclei; and he further ascertained that the mother-cells of the spores have only four chromosomes in their nuclei, so that a reduction by half of the number of the nuclear chromosomes must take place in these cells. The mother-cells of the spores increase considerably in size previously to division, and at the same time become tetrahedrally four-lobed. Between the lobes, septa grow inwards towards the centre of the cavity; and a four-poled spindle is

[1] Studies in Hepaticae: On *Pallavicinia decipiens*, Mitten, Annals of Botany, Vol. viii, No. XXIX, March, 1894.

formed about the nucleus, each pole corresponding in position to a lobe of the cell. The four chromosomes now become differentiated in the nucleus of the spore-mother-cell, and undergo, as Farmer believes himself to be justified in asserting, double longitudinal splitting; four chromosomes then wander into each developing spore: finally, the septa separating the four spores are completed. The same processes take place, according to Farmer, in the spore-mother-cells of *Aneura*. Photographs of the preparations, which I owe to the kindness of the author, bear out the correctness of his statements.

It may appear superfluous to enter upon further speculations how the relations in the number of the chromosomes present themselves in the lower Cryptogams, the Fungi and Algae. I will, however, venture to formulate the problem which has to be solved with reference to these plants, in the hope of giving a stimulus to the necessary research. As a matter of fact, no countings of the chromosomes in the dividing nuclei in the lower Cryptogams have been undertaken: this is due partly to the great difficulty with which counting is attended, and partly to a lack of appreciation of the importance of these countings. The first question to be asked is, whether in these lower Cryptogams, in which a true alternation of diverse sexual and asexual generations does not take place, the number of the chromosomes in the nucleus is at all determinate; and if it be so, whether and when a reduction takes place of the double number of chromosomes resulting from a sexual act. With regard, in the first place, to the question whether or not there is a determinate number of chromosomes in Algae and Fungi, I am inclined to answer it in the affirmative: for I was able to count twelve chromosomes in the nuclear discs of *Spirogyra polyteniata*[1], which number was also determined by J. W. Moll in *Spirogyra crassa*[2]; and I am almost certain of the uniformity in number of the chromosomes in *Trichia fallax*, an organism belonging

[1] Kern- und Zell-Theilung, p. 11: Histol. Beitr. I, 1888.

[2] Observations on Karyokinesis in *Spirogyra*, Verh. d. Kon. Akad. van Wetensch. te Amsterdam, Tweede Sectie, Deel I, No. IX, 1893, p. 29.

to so low a group as the Myxomycetes. I believe that the nuclei of *Trichia fallax* contain each twelve chromosomes: I counted them in my old preparations[1] showing numerous nuclear divisions in developing sporangia. It is true that the nuclei are so small that absolute accuracy in the counting is hardly attainable: still one cannot but be impressed by the remarkably uniform appearance of the nuclear divisions. If, however, the number of the chromosomes be constant in the Myxomycetes, there can be little doubt but that it is so universally: and then it becomes probable that a reduction in the number of the chromosomes must be associated with some definite developmental stage in those of these lower Cryptogams which are sexually differentiated. The assumption that this reduction takes place during the development of the sexual organs is not supported by any direct evidence, and it is contrary to what has been ascertained in the higher Cryptogams. It may be that the reduction follows the sexual act. In all these cases in which the original generation is directly developed from the product of fertilization, the reduction probably takes place during germination: here the zygote is all that represents that developmental stage, the asexual generation, which intervenes in the Muscineae, the Pteridophyta, and the Phanerogams, between fertilization and formation of spore-mother-cells. On the other hand it is possible that, in *Coleochaete*, *Oedogonium*, or the Florideae, the reduction does not take place until the development of the, motile or non-motile, spores with which the product of the sexual act closes its existence; for it is from these spores that the first generation is, in turn, developed.

The constancy of the number of the chromosomes in the nuclei of the sexual cells is doubtless of great importance, for it ensures the equal influence of the two parents in the sexual act: and the act of fertilization is, in all the higher organisms, the centre of gravity of the maintenance and development of the species. In contrast with this is the

[1] Zur Entwicklungsgeschichte der Sporangien von *Trichia fallax*, Bot. Zeitg., 1884, Taf. III, Fig. 6.

fact that the number of the chromosomes in the nuclei of the somatic cells of both the sexual and the asexual generations has been found to vary. But, so far as my experience goes, these variations are always to be observed in the nuclei of cells which are not longer embryonic, like those in an embryo or in a growing-point, but which, on the contrary, are to some extent histologically specialized and are not destined to eventually give rise to reproductive cells. Both Guignard and I have often observed variation in the number of the chromosomes in the cells of the nucellus and integuments of the ovules. The determinate number of chromosomes is still more frequently departed from in nuclei which are definitively excluded from the sphere of reproduction. Thus Guignard[1] found in species of *Lilium* that the lower nucleus in the embryo-sac, from which the antipodal cells are derived, has not twelve chromosomes like the upper nucleus which gives rise to the egg-apparatus, but sixteen, twenty, or even twenty-four chromosomes. The secondary nucleus of the embryo-sac, by the division of which the development of the endosperm is initiated in the Angiosperms, is produced by the fusion of the two (upper and lower) polar nuclei, and must therefore contain as many chromosomes as both the polar nuclei. Hence, in *Lilium*, the nuclei of the endosperm are usually found to contain more than twenty-four chromosomes, although it represents the generation which typically possesses only twelve chromosomes in each nucleus. Some time ago[2] I described the frequent nuclear fusions which gradually take place in the developing endosperm of the Angiosperms when, as the cell-areas are being marked out, each area encloses several nuclei. As regards the Gymnosperms, Dixon was able to ascertain that, in *Pinus sylvestris*, the determinate number of chromosomes was adhered to in the prothallial nuclei of the embryo-sac, until the development of the archegonia: but when the development of the archegonia is initiated, the determinate

[1] Nouvelles Études, p. 187.

[2] See especially, Zellbildung und Zelltheilung, 3. Aufl., 1880, p. 25.

number of chromosomes may be departed from in the other prothallial cells without any possible prejudice to the reproductive processes, and accordingly the number is found to increase, or even to double itself, in the large nuclei of the cells forming the walls of the archegonia [1].

What has just been stated suffices to prove that variations from the determinate number of chromosomes are possible. Similar variations have also been observed among animals, but I will not discuss them as I am not in a position to estimate their significance [2]. Among the examples from the plant-kingdom which have been cited, that of the lower nucleus in the embryo-sac of the Lilies, so carefully studied by Guignard [3], appears to be the most instructive. This nucleus has originally twelve chromosomes, but in the next prophase a larger number can be detected. From this it might naturally be inferred that the reduction in the number of the chromosomes as exhibited in the sexual cells does not call for any phylogenetic explanation, and that it is superfluous to regard it as a reversion to an older condition of things, since a change in the number of the chromosomes may take place quite independently of any such assumption. But the variation is essentially different in the two cases. The change in the number of the chromosomes which is associated with the alternation of generations is accompanied by other deep-seated changes, which can be detected in the altered appearance of the spore-mother-cells. Moreover the change in the number of the chromosomes associated with the alternation of generations gives rise, not to a variable, but to a perfectly constant result, which can only be attributed to phylogenetic causes. The purely vegetative—they may be almost called accidental—variations in the number of the chromosomes within the limits of any one generation, do not otherwise affect the appearance of the nuclei, and the re-

[1] Dixon, loc. cit., p. 32.

[2] Compare especially Valentin Haecker, Ueb. generative und embryonale Mitosen, &c., Arch. f. mikr. Anat., 43, p. 773, 1894.

[3] Loc. cit., p. 187.

sulting number is quite indeterminate. Thus, in the lower nucleus in the embryo-sac of the Lilies, from twelve to twenty-four chromosomes make their appearance in the prophase. The lower nucleus is larger than the upper one, though in other respects similar; and it may be that the increase in number of the chromosomes in the lower nucleus is a definite result of more ample nutrition, and the same influences may be at work in those cases of apogamy in which the number of chromosomes characteristic of the asexual generation is attained in a purely asexual manner. The case of the adventitious development of embryos in Phanerogams is not one that offers any difficulty: for the cells of the nucellar tissue from which the embryos spring already contain as many chromosomes as does the fertilized ovum. But the case of Fern-prothallia from which the cormophytic asexual generation is developed as a bud, is altogether different. The nuclei of the prothallial cells contain only half as many chromosomes as do the cells of the asexual generation: hence it is probable that on the development of the growing-points of the asexual generation, the number of the chromosomes in the nuclei is doubled. Overton, who has already dealt with this problem from the theoretical point of view, is of opinion that it presents no greater difficulty than does parthenogenesis, and he draws attention to the fact that the lower nucleus in the embryo-sac of *Lilium* changes the number of its chromosomes quite independently[1]. Direct observation alone can decide whether the number of chromosomes in the nuclei of an apogamously developed Fern is increased independently; or whether, though I do not regard the suggestion as probable, its nuclei have the same number of chromosomes as those of the prothallium. If the latter be the case, then the development of the spores of these plants is not attended with a reduction in the number of the chromosomes. The assumption that a doubling of the number of the chromosomes takes place, under the influence of correlative processes, in the apogamous development of

[1] Loc. cit., pp. 14, 15, of the separate copy.

a Fern, is supported by the fact that apospory also occurs among Ferns. In certain varieties of *Athyrium*, *Polystichum*, and *Aspidium*, as F. O. Bower has shown [1], fertile prothallia are developed in place of sporangia. It would appear, therefore, that the nuclei of these prothallia must contain twice as many chromosomes as do those of normally developed prothallia; and consequently, since no reduction in the number of the chromosomes occurs in connexion with the development of the sexual cells, these cells would, in this special case, contain twice the normal number of chromosomes. It is, however, more reasonable to assume, until direct observation proves the contrary, that the aposporous development is attended with a correlative reduction in the number of the chromosomes. On similar grounds it is probable that a reduction also attends the development of protonema, eventually bearing sexual plants, which has been induced in the sporogonia of certain Mosses. From the fragments of setae of species of *Hypnum* and *Bryum*, Pringsheim [2] obtained the development of protonema; as did also Stahl [3] with *Ceratodon purpureus*, not only from cells of the seta but also from those of the wall of the capsule. It is probable that in this protonema a reduction of the number of the chromosomes takes place under the influence of correlative processes.

The foregoing phenomena suggest the raising anew of the question as to the continued independent existence of the chromosomes. In the case of plants it may not be considered as settled that, in the resting nucleus, the chromosomes present no free ends. Guignard [4], whose statements are perfectly correct [5], found but a single filament at the beginning of the prophase in the nuclei which he examined.

[1] On Apospory and allied phenomena, Trans. Linn. Soc., Ser. Bot., Vol. ii, Part 14, p. 301, 1887.

[2] Ueb. vegetative Sprossung der Moosfrüchte; Monatsber. d. Berl. Akad. d. Wiss., June 10, 1876.

[3] Ueb. künstlich hervorgerufene Protonemabildung an den Sporogonien der Laubmoose, 1876.

[4] Nouvelles Études, p. 253.

[5] Strasburger, Schwärmsporen, Gameten, &c., Histol. Beitr., Heft IV, 1892, p. 147.

This filament breaks up into a given number of segments simultaneously, not by successive divisions into two: it is on this account that such numbers as twelve are frequently found, numbers which cannot be the result of repeated division into two. The fact that, as a rule, the same number of chromosomes occurs in successive generations of nuclei, suggests the view that though the chromosomes may lose their morphological individuality in the resting nucleus, they do not lose their physiological individuality. The observation of such a series of stages of nuclear division as can be obtained by the laying open of embryo-sacs in which the development of the endosperm is commencing, makes it difficult to resist the impression that it is always the same chromosomes which make their appearance over and over again in the repeated divisions. In the prophase the chromosomes are seen to appear in precisely the same positions as they occupied in the preceding anaphase: and if the picture of the anaphase were proportionately enlarged it would exactly correspond to that of the succeeding prophase. In one word, it must be assumed that the individuality of the chromosomes persists in the resting nucleus, and determines the breaking up of the nuclear filament into the corresponding number of chromosomes in the succeeding prophase. Any change in the number of the chromosomes must be preceded by an alternation, whether increase or diminution, in the number of the chromosomatic individualities. The reduction of the number of the chromosomes by half, at the initiation of the sexual generation, is due to the fusion into one of two chromosomatic individuals, under the influence of causes which, for the present, can only be assumed on phylogenetic grounds. These fusions of chromosomes, at the initiation of the sexual generation, can apparently only take place under certain conditions. They are affected by abnormal internal changes: for instance, the embryonic substance constituting the growing-points of shoots affected with bud-variation often remains sterile; and hybridization frequently induces similar consequences.

My developmental studies on the spermatozoids of plants [1] impressed me with the conviction that the surrender of morphological individuality by no means involves, for these chromosomes, the loss of physiological individuality. It is only on the assumption of the persistence of this physiological individuality that it is possible to account for the fact that a homogeneous filament in the nucleus of a spermatozoid gives rise, in the fertilized ovum, to a predetermined number of chromosomes.

It is established that, in the higher plants, all the nuclear divisions which lead up to the formation of the sexual cells are normally attended by longitudinal splitting of the chromosomes, so that the number of the chromosomes remains the same throughout. There is no such thing, among plants, as nuclear divisions resulting in the reduction by one-half of the number of the chromosomes. Such a conception involves the assumption that the entire, not longitudinally split, chromosomes of the mother-nucleus become separated into two groups, each of which goes to form a daughter-nucleus [2]. If this be so, then each daughter-nucleus must contain only half as many chromosomes as the mother-nucleus; and, in the next generation, each nucleus must contain only half as many chromosomes as a daughter-nucleus: but nothing of the kind can be observed among plants, a fact which has to be taken into account in a consideration of the phenomena of heredity. Among animals too, as is shown by recent researches, the division with reduction taking place in the mother-cells of ovum and spermatozoon is dependent upon previous longitudinal splitting of the chromosomes, and is therefore referable to normal nuclear division: and even were there not sufficient evidence to prove this, the facts are so clearly ascertained with regard to plants, in which the phenomena of heredity and variation are essentially the same

[1] Schwärmsporen, Gameten, &c., p. 145.

[2] Weismann, Ueb. die Zahl der Richtungskörper und ihre Bedeutung für die Vererbung, p. 79, 1894.

as in animals, that all possibility of misinterpretation is excluded, and that their importance cannot be overlooked [1].

Just as the facts ascertained among plants exclude the assumption of nuclear division with reduction, so also do the observations on nuclear division in plants give no support to the view that karyokinesis is ever attended with hereditarily unequal division, and, so far as my information goes, the observations made on animals are likewise unfavourable to this view. Ever since an accurate knowledge of the longitudinal splitting of the chromosomes during nuclear division, and of the equal distribution of the products of this splitting, was attained, I have become more and more firmly convinced that the object of the process is the qualitatively equal division of the chromosomes. Theoretical speculation, which transcends the limits of experience, must start from definitely ascertained facts. Minute investigation of the longitudinal splitting of the chromosomes can but produce the impression of equal division: there is absolutely no foundation in fact for the assumption of unequal division. Hence, from the very beginning, I have taken the standpoint of epigenesis in forming my theoretical interpretation of the facts of development [2]. The only conception of development that I am able to form is that it is a succession of stages, such that each stage determines the conditions for the succeeding stage and inevitably leads on to it. In my opinion, development belongs to the category of correlative processes, and can only be comprehended from this point of view. The cell-nuclei, in whatever part of the body they may be, are and remain

[1] See Boveri, Zellen-Studien, Heft I, 1887, p. 13 ff., 77, and Heft III, 1890, p. 51: also August Brauer, Ueb. das Ei von *Branchippus Grubii* von der Bildung bis zur Ablage, in Anhang zu den Arbeiten der Akad. d. Wiss. zu Berlin, 1892: O. Hertwig also admits the possibility of the reference of division with reduction to normal nuclear division in his Ei- und Samenbildung bei den Nematoden, Arch. f. mikr. Anat., Bd. 36, 1890, pp. 65 ff. of the separate copy.

Also Valentin Haecker, Ueb. Generation und Embryosack-Mitosen, &c., Arch. f. mikr. Anat., Bd. 43, 1894, p. 759: see also my work, Schwärmsporen, Gameten, &c., p. 151.

[2] See, Das Protoplasma und die Reizbarkeit, 1891, pp. 20, 27.

endowed with all the characteristics of the species; but their activity is stimulated in a definite direction by the prevalent conditions. Were this not the case, it would be impossible for the renewed development of organs to take place, as it does, from any part of the plant-body, organs which manifest all the characteristics of the species; nor would it be possible to stimulate certain activities by artificial interference, and to induce this or that manifestation of hereditary capabilities. It is in this way that I account for the influence of those external conditions, for instance, which determine sexual or asexual reproduction in the Algae; as also for the influence of certain substances, formed by the organism itself, which induce the formation of a flower at the growing-point.

I have rejected the view of the hereditarily unequal division of nuclei on the ground that it is contrary to the facts ascertained by direct observation, and I am equally unable to admit that theories of heredity are justified in reconstructing the nucleus with the object of finding in it all the structures which are necessary to them: the only legitimate point of departure is afforded by the actually observed facts of nuclear structure. I consider Weismann's conception of the id [1], as an element in the nucleus which is charged with all the hereditary characteristics of the species, to be felicitous, because it appears to me that it can be supported by direct observation. I regard as ids the discoid segments of the chromosomes, which are all exactly similar in form and structure, and are serially arranged with such remarkable regularity in the chromosomes of nuclei about to divide. Whilst in the resting-stage of the nucleus the substance of each id is distributed, for nutritive purposes, over the elongated nuclear filament, in the prophase the substance segregates to constitute and form a segment of the series. In the id there are represented not only the small chromatin-

[1] Das Keimplasma, eine Theorie der Vererbung, p. 84: p. 60 in the English edition, 1893.

granules previously distributed in the linin-network, but also, and perhaps to the largest extent, the linin-network itself. For it is well known that the staining capacity of the contents of the nucleus increases considerably in the prophase, passing over for the most part into that readily stainable condition which we regard as an increase of the chromatin; in the anaphase the nuclear contents undergo precisely contrary changes. In support of the view that the individual segments present in the chromosomes of nuclei about to divide, possess all the hereditary characteristics of the species and are, in fact, the true ids, may be adduced the results of the microscopic vivisection of unicellular organisms[1]: it has been found, namely, that a fragment of such an organism will regenerate itself to a complete individual if only it contains a portion of the nucleus. Again, I have observed that when, as not infrequently happens during the division of the pollen-mother-cells of *Hemerocallis fulva*, single chromosomes do not enter into the structure of either of the daughter-nuclei, but remain behind in the equatorial plane of the nuclear spindle, small pollen-grains are formed in relation with them. The small chromosome marks itself off from its surroundings, and a proportionate mass of the cytoplasm of the mother-cell is assigned to it[2]. The often very small pollen-grain develops quite normally and shows all the peculiarities of structure characteristic of the species.

The serially arranged ids in the chromosome are, in my opinion, repetitions of each other, for no difference can be detected between them by actual observation. It is possible to go on to assume that they are repetitions which correspond to successive generations, and that they actually represent, as Weismann puts it, ancestral plasm. It is by their simultaneous activity that the constancy of the species is proportionately maintained: for the co-operation of so many ids

[1] See especially A. Gruber, Mikroskopische Vivisection. Ber. d. naturf. Ges. in Freiburg im Br., Bd. VII, Heft I.

[2] Ueb. den Theilungsvorgang der Zellkerne, 1882, p. 20, and Taf. II, Figs. 63–65.

must produce a resultant effect which would be a mean between the individual variations of the successive generations. If, however, in consequence of the repeated union of individuals presenting a similar variation, the number of ids representing this variation be increased, the variation must become permanent.

At each longitudinal splitting of the chromosomes during nuclear division, all the ids are halved and are equally distributed to the succeeding generations of nuclei. The number of the ids would, however, become doubled at each sexual act, were it not for the reduction which takes place at the initiation of each sexual generation. Since this reduction is not due either to extrusion or to an absorption of the chromosomes, at least in plants, the only remaining explanation is that it is due to the fusion in pairs of the ids and therefore also of the chromosomes. In the processes of differentiation which take place in the nucleus of a spore-mother-cell during the prophase, the substance of each pair of ids aggregates into a single id. In this way the idioplasm of many and different ancestors enters into the formation of each individual id. I do not, however, consider that these ancestral plasms exist isolated in the id; I regard them as completely fused into one. The number of the ids is doubtless, like that of the chromosomes, hereditarily determined: but the numbers in different organisms certainly do not stand in any definite relation to each other, for even closely allied species of plants, which have ids of apparently the same size, sometimes present different numbers of chromosomes: for instance, in the Liliaceae the nuclei of the spore-mother-cells contain, according to the species, 8, 12, 16, or 24 chromosomes. It would appear, therefore, that the number of the chromosomes possesses no deep significance: and do not the two externally indistinguishable varieties of *Ascaris megalocephala*, which have been so fully investigated, differ in that the nuclei of the one contain only half as many chromosomes as do those of the other?

It is now known, and the point has been made especially

clear by observations on *Ascaris nigrovenosa*[1], that the chromosomes of the two parents do not lose their independence in connexion with the sexual act. In this *Ascaris* both the sperm-nucleus and the germ-nucleus independently go through the prophases of division, and it is only then that the two sets of chromosomes arrange themselves in the common spindle of the embryonic nucleus. In each subsequent nuclear division each half contains a number of chromosomes identical with that of the combined parental chromosomes in the fertilized ovum. Hence in hybrids the chromosomes of both father and mother continue active; and the behaviour of hybrids shows peculiarities which are very instructive for the comprehension of the phenomena of heredity in the offspring of legitimate unions. Hybrids may exhibit in all their parts a combination of the characters of the two parents; or they may show this only in certain parts, whilst other parts present the distinct characters of one or other of the parents; or they, on the whole, resemble one parent more than the other; or, finally, they may altogether resemble one of the parents. Naudin[2] has drawn attention to the fact that, in some hybrids, the characters of the two parents, instead of being blended, are manifested in patches; this may occur in all parts of the plant, but it is especially marked in flowers and fruits. In such a case the hybrid is a sort of mosaic made up of portions of the two parents. Millardet[3] has recently given an account of hybrids which are more like, or, in the extreme case, exactly resemble either the father or the mother.

The hybrids of mosaic-like constitution may perhaps be adduced as evidence in support of the possibility of hereditarily unequal division of the nucleus; and one of the cases described by Millardet, that of a hybrid Vine, known as the

[1] Edouard van Beneden, Rech. sur la maturation de l'œuf, &c., Taf. XIX, *bis et ter.*

[2] Sur l'hybridité dans les végétaux, Nouv. Arch. du Muséum, I, 1865, pp. 33, 49, 151.

[3] Note sur l'hybridation sans croisement, ou fausse hybridation, Mém. de la Soc. d. Sci. phys. et nat. de Bordeaux, t. IV, sér. 4, 1894.

York-Madeira, would lend itself to this purpose. This hybrid appears to be the offspring of the spontaneous crossing of *Vitis aestivalis* and *V. Labrusca*. On the under side of its leaves it presents not only the sunk stomata of *V. aestivalis* and the projecting stomata of *V. Labrusca*, but also every possible intermediate form of stoma. From these facts the inference might be drawn that the epidermis of the leaf of this plant consists of cells which belong to the type of the father, or to that of the mother, or to an intermediate type. If this be so, the type must be manifested in the individual cells, since the two guard-cells of a stoma are developed from a single mother-cell. If now this case is to be explained by referring it to a difference in the nuclei of these cells, induced by hereditarily unequal division, the attempt might appear plausible enough: but such an assumption would be directly contradicted by those cases in which the hybrid entirely resembles either the father or the mother, cases which have been observed not only in the genus *Vitis*, but also in *Rubus* and *Fragaria*. For in these latter cases it is an inevitable consequence of the theory of hereditarily unequal division, that somewhere in the body of the hybrid there must be a preponderance in favour of the parent which the hybrid does not resemble; but, as a matter of fact, this does not occur. Hence the only possible explanation seems to me to be that the interaction of the chromosomes in the nucleus gives rise to phenomena of interference: in those hybrids which entirely resemble either the father or the mother, it may be assumed that the influence of the chromosomes of the one parent is completely neutralized by that of the chromosomes of the other; whereas in other hybrids some characteristics are weakened, whilst others are strengthened, by interference. Similarly, just as in hybrids, so the offspring of parents of the same species may either combine the characters of the parents, or resemble one or the other more strongly.

Atavistic phenomena clearly prove that the ids whose action is neutralized are not absorbed or otherwise destroyed. A very instructive case illustrating this point is that of the

peloric Snapdragon (*Antirrhinum majus*) described by Charles Darwin[1]. The seed produced by the peloric plants when fertilized with their own pollen yielded only peloric individuals: whilst the seed produced by peloric plants fertilized with the pollen of the normal forms yielded only normal plants; and similarly the seed produced by plants of the normal form fertilized with pollen from the peloric form yielded only normal plants. Hence the influence of those chromosomes which induced the peloric condition was, in the two latter cases, neutralized by the chromosomes of the normal form. But the peloric chromosomes were not destroyed, for the descendants of the normal plants of semi-peloric origin were peloric to the extent of one-third.

However peculiar may be the mixture of the parental characters which a hybrid presents, it is repeated in all hybrids having the same origin. But this is not the case with the offspring of hybrids fertilized with their own pollen: on the contrary, the progeny is now remarkable for a high degree of variability. In successive generations resulting from repeated fertilizations with their own pollen, there is a growing tendency to revert to the parental forms. But few hybrids, fertilized with their own pollen, continue to reproduce themselves unchanged, and thus practically constitute new species. Weismann[2] endeavours to account for the variability of the offspring of hybrids by referring it to divisions accompanied with reduction, in the development of the sexual cells: these reducing divisions result in dissimilar products, and the union of the dissimilar products induces variability. This explanation is, however, inadmissible because, as a matter of fact, such divisions do not take place among plants: the causes of the variability of hybrids must be sought elsewhere. They are to be looked for in those processes which lead to

[1] Das Variiren der Thiere und Pflanzen im Zustande der Domestication, Germ. ed., 1868, Bd. II, p. 92: Variation of Animals and Plants under Domestication, ii, pp. 70, 93, 1868.

[2] Das Keimplasma, eine Theorie der Vererbung, 1892, p. 293: Engl. Ed., p. 299.

the reduction of the number of chromosomes in the mother-cells of the spores. Hybrids of similar origin resemble each other, in the first generation, because the chromosomes of both parents persist side by side in all the nuclei of these hybrids, and affect the processes of development in a definite manner. The offspring of these hybrids behave differently, doubtless because there is a fusion of the parental chromosomes and a corresponding reduction in the number of the ids in the process of development of the spore-mother-cells (mother-cells of the pollen and of the embryo-sac). Hence interference becomes more active, and renders possible a difference in the sexual cells; and the union of these diverse cells in the sexual act is the starting-point of diversity in the progeny. It is conceivable that this diversity is due to the influence of isolated ids, of ids derived from one or other of the original parents, but remaining unfused. It may be further suggested that the continued production of unchanged progeny by hybrids is only possible in those cases in which the chromosomes and the ids of the original parents retain their primitive equivalence even after reduction and fusion have taken place.

The process of reduction of the number of the chromosomes by half takes place, in the Muscineae, Pteridophyta, and Phanerogamia, in the spore-mother-cells, that is, at the close of the generation developed from the fertilized ovum; but in the lower Cryptogams, where the cell produced by the sexual act does not give rise to a definite organism representing the asexual generation, the reduction probably takes place on the germination of this cell. The attempt has been made to give a phylogenetic explanation of this reduction in the number of the chromosomes, and it has been regarded as a reversion of ontogeny to the point of origin. The phenomenon under consideration is essentially that of the return of the most highly organized plants, at the close of their life-cycle, to the unicellular condition: in one word, it is the repetition of phylogeny in ontogeny. This explanation seems to me to be likewise the only one admissible in the case of animals. It is, however, an altogether different question, whether or not the

double division which takes place in the mother-cells of the spermatozoa, and of the ova in connexion with the development of the sexual cells in animals, may not be interpreted as indicating the existence of a distinct generation. The remarkable uniformity presented by the double divisions in the development of the sexual cells of animals is unfavourable to the assumption that the product of the double division represents what remains of a once independent sexual generation. Were this the case, it is certain that the reduction would be manifested in different degrees in the various sub-divisions of the animal kingdom. It may, with greater probability, be assumed that, in all those sub-divisions of the animal kingdom, sexual differentiation occurred at the very beginning of phylogenetic development, and that the product phylogenetically developed from the sexual act is the direct continuation of the ontogeny of the individual. The process of reduction which takes place in the mother-cells of ova and spermatozoa of animals is associated, as in plants, with far-reaching changes[1]; and the abundance of chromatin in these altered nuclei may, as also in plants, induce a rapid division into four of the nuclei. In plants this division into four takes place in the spore-mother-cells, without any direct relation to the sexual cells. That the constitution of the mother-nuclei induces the two divisions which rapidly follow each other, is shown by the fact that the mother-cells of the ova in animals give rise, on division, to products of unequal size: hence the subsequent division into four cannot be attributed to the form of the mother-cell. The division into four of the so-called paranuclei of the Infusoria doubtless takes place in relation with a corresponding reducing process[2], although under conditions which differ from those obtaining in the conjugating Infusoria. The uniformity in the phenomena of reduction and in the processes

[1] Compare O. Hertwig, Lehrbuch der Entwicklungsgeschichte, 4. Aufl., 1893, p. 32.

[2] Compare O. Hertwig, Die Zelle und das Gewebe, 1893, p. 216, where the literature is cited.

of division exhibited by the mother-cells of the ova and spermatozoa of those animals in which they have been minutely investigated, doubtless depends upon homology: the divisions into four after the process of reduction must have a common cause. The analogy is very striking between the much-discussed processes of splitting of the chromosomes in the mother-nuclei of animals, and the processes described by Farmer as taking place in the spore-mother-cells of Liverworts: for in *Pallavicinia* and in *Aneura* the number of chromosomes requisite for the two following divisions is provided at one and the same time in the nucleus of the spore-mother-cell. In these Liverworts, indeed, the abbreviation is the more marked, since a quadripolar nuclear spindle is formed, and the products of the longitudinal splitting of the chromosomes are simultaneously distributed to the four daughter-nuclei. It is of special purpose that I cite this particular case, for it alone, in plants, corresponds to the phenomena observed in animal ova: in these plants, as in animals, the double division follows on the process of reduction, and introduces the diminished number of chromosomes characteristic of the sexual cells. It must, however, be pointed out that the nuclear divisions with multipolar spindles to be found in the developing endosperm[1] of Angiosperms and in pathogenic tissues of animals, are not comparable with those here alluded to. For in those cases the division is not preceded by any internal changes in the nucleus; the number of the poles is variable, even accidental; and the number of the chromosomes distributed to the daughter-nuclei is not always the same.

I have already suggested that, in cases in which the product of the sexual act directly gives rise to the original generation, a process of reduction taking place at the commencement of germination is to be anticipated. It is possible to interpret in this sense the observations of Klebahn and Chmielewsky on the process of the germinating zygotes of

[1] Zellbildung und Zelltheilung, 3. Aufl., 1880, p. 18.

certain conjugate Algae. Klebahn [1] found that, in the germinating zygotes of *Closterium* and *Cosmarium*, the nucleus divides twice in rapid succession; but the zygote then divides into only two cells, and only one nucleus for each of these two persists. Similarly, according to Chmielewsky [2], the nucleus of the zygote of *Spirogyra* divides into four, but the unicellular embryo subsequently possesses but a single nucleus. Klebahn has already suggested that, in these cases, a reduction in the number of the elements, probably by fusion with one another, must necessarily take place [3]: and Chmielewsky compares the phenomena in *Spirogyra* with the formation of polar bodies, a comparison to which Klebahn offers some objections [4], since the assumption of the formation of polar bodies after a sexual act would involve a modification of prevalent views as to the nature of these structures. Oscar Hertwig [5] considers 'that the processes described by Klebahn in the zygotes of Desmids, have the same object as the reducing divisions during the maturation of ova and spermatozoa.' 'As in that case the double division of the nucleus involves a reduction by half of the normal nuclear substance, before fertilization, and thus prevents an accumulation of nuclear substance which would otherwise take place as a consequence of the fusion of two nuclei in the sexual act; so, in the Desmids, it would appear as if it were only after conjugation that a reduction of the nuclear substance is eventually effected, and thus the doubled mass of the nucleus in consequence of the fusion of two complete nuclei were brought back to the normal. The nucleus of the zygote, instead of dividing once, is divided into four by two immediately successive divisions;

[1] Studien üb. Zygoten: I, Die Keimung von *Closterium* und *Cosmarium*, Jahrb. f. wiss. Bot., XXII, p. 415, 1891.

[2] The paper is in Russian, but a full abstract of it is given in Famintzin's Uebersicht der Leistungen auf dem Gebiete der Botanik in Russland während des Jahres 1890, p. 16.

[3] Loc. cit., p. 441.

[4] Studien üb. Zygoten: II, Die Befruchtung von *Oedogonium Boscii*, Jahrb. f. wiss. Bot., XXIV, p. 257. 1892.

[5] Die Zelle und das Gewebe, 1893, p. 225.

but the cytoplasm divides but once, and each half receives only one functional nucleus, whilst the remaining two nuclei, being superfluous, become disorganized.' O. Hertwig thus compares the processes occurring in these zygotes with the reducing divisions in the mother-cells of the ova and spermatozoa of animals which he defines as follows[1]: 'The essential feature of these divisions is that two closely related divisions follow each other in immediate succession, such that the second succeeds the first without an intervening resting-stage of the nucleus Consequently the groups of nuclear segments resulting from the first division are immediately divided, without previous longitudinal splitting, into two daughter-groups. At the close of the second division the ripe ovum or spermatozoon receives only half so many nuclear segments, and, consequently, only half so much nuclein, as do the nuclei formed in an ordinary division in the same animal.' O. Hertwig thinks[2] that accurate counting of the nuclear segments in the various stages of division, would confirm his view that a reducing division takes place in the zygotes of the Desmids. If, however, my interpretation of the process is correct, then the reduction in the number of the chromosomes must take place at the commencement of the prophase of the nucleus of the zygote, and, as in so many other cases, its rapid division into four would be the consequence of changes undergone during the process of reduction.

When the morphological value of the polar bodies of the ova of animals is correctly estimated, and when the comparisons between the generative processes of animals and those of plants are accurately drawn, it becomes at once apparent how little justification there was for the attempt to find, in connexion with the ova of plants, structures which should correspond to the polar bodies of animals; and how erroneous it was to regard the ventral canal-cells of Vascular Cryptogams and Gymnosperms as structures of this nature. This con-

[1] Loc. cit., p. 189: see also Vergleich der Ei- und Samenbildung bei Nematoden, p. 65 of the separate copy.

[2] Loc. cit., p. 225.

sideration shows yet once more to how great an extent the advance of theoretical comprehension affects the correct apprehension of the problem to be solved, and how this correct apprehension may save a great deal of superfluous labour.

The reduction in number of the chromosomes takes place, among the higher plants, in the mother-cells of the spores, and it is consequently these which must be regarded as the first term of the new generation. They assert this their true significance in that they usually isolate themselves from cohesion with other cells and become independent, although this independence is only of practical utility in the case of the products of their division, that is, of the spores. Hence the centre of gravity of the developmental processes which take place in both micro- and macro-sporangia of Cryptogams and Phanerogams, does not lie in those cells, cell-rows, or cell-aggregates, which give rise to the sporogenous tissue and have been designated 'archesporium' by Goebel[1]. The archesporium still belongs to the sexually-developed asexual generation; it is only the spore-mother-cells which initiate the new sexual generation: consequently the presence or absence of a well-defined archesporium is not a matter to which importance should be attached. For the archesporium is merely the merismatic tissue from which the spore-mother-cells are derived, a tissue which is frequently, but by no means necessarily, differentiated from the surrounding tissues at an early stage; so that its differentiation cannot be of fundamental importance.

[1] Vergl. Entwicklungsgeschichte der Pflanzenorgane, 1883, p. 284.

Geotropic Sensitiveness of the Root-tip [1].

BY

W. PFEFFER.

A RADICLE placed horizontally bends, as is well known, until its tip points vertically downwards, that is until it has attained its normal position of equilibrium. This geotropic movement is occasioned by gravitation, which however merely operates as a stimulus; that is, it affords the impulse in obedience to which the plant executes the necessary movement of curvature by means of the factors of growth at its command.

In principle then, the case is something like that of a man in darkness to whom a single lingering ray of light gives in the same way the impulse to so change his movements as to make his way towards the light. It is plain that the processes involved in the movement by which such an act is accomplished are to be distinguished from the perception of the stimulus. Furthermore, as is well known in the case of the higher organisms, and as also frequently occurs in plants the sensitive organ may be separated by some distance from the organs that perform the external action.

Such a relation exists in the root, in which the tip alone is geotropically sensitive. The root-tip therefore is the part

[1] Preliminary Communication. Read before Section D of the British Association, Oxford, August 11, 1894.

[Annals of Botany, Vol. VIII. No. XXXI. September, 1894.]

that perceives the geotropic stimulus, and, as a result, enables the adjacent parts, that are themselves not sensitive, to carry out the geotropic curvature.

The geotropic reaction of the root was first conceived of in this way by Ch. Darwin [1] in the year 1880. Absolute proof of this view, to be sure, was not obtained by Darwin, nor has it been by the studies of other investigators [2]. As a matter of fact, however, Darwin's assumption is right, as is irrefutably proved by investigations that have recently been conducted under my direction by Dr. Czapek.

All previous investigations have been inconclusive because they were judged of by the results that followed cutting off the tip of the root. But by the infliction of such a wound the previous capacity for reaction may be suspended or destroyed. If then the root, after removal of the tip, no longer reacts geotropically, it is still wholly undetermined whether this result is occasioned by the want of the sensitive root-tip, or by the fact that through the infliction of the wound the geotropic sensitiveness has been suspended.

That such a suspension of the capacity for reaction may occur is shown very clearly by investigations on heliotropic sensitiveness which Professor Rothert [3] conducted in the Leipsic botanical institute. It was found, among other things, that the young cotyledon of *Avena sativa* is heliotropically sensitive throughout, but especially so at the tip. An heliotropic curvature of the uninjured leaf follows, therefore, as well when the tip alone, as when the middle of the leaf alone, is exposed to light. But if a small piece is cut off from the leaf-tip, the leaf becomes indifferent to illumination from one side, and only after one or two days, that is after the reaction due to wounding has ceased, does the sensitiveness return.

[1] The Power of Movement in Plants, 1880, p. 523.

[2] Among those who have studied the question are Detlefsen, Wiesner, Fr. Darwin, Kirchner, Krabbe, Brunchorst, Fritsch. The literature is given by Brunchorst, Berichte d. deutsch. botan. Gesellschaft, 1884, p. 79.

[3] Berichte d. deutsch. botan. Gesellschaft, 1893, p. 387.

But with this object it is possible to demonstrate with certainty that only the capacity to perceive the stimulus has been lost by wounding, but not the capacity to conduct a stimulus already perceived. For if we expose the tip of a leaf to light for only a short time, and cut it off before curvature is noticed, the heliotropic reaction takes place in the wounded leaf precisely as in an uninjured one, although through wounding the sensitiveness of the leaf has been suspended.

In an entirely analogous way, a reaction manifested in geotropic curvature takes place in roots when they are decapitated after geotropic induction. That, however, only proves that an actual geotropic induction is not necessarily connected with the continual existence of sensitiveness. The attempts made to prove from this fact the sensitiveness of the root-tip are in so far based on incorrect inferences.

As regards heliotropism the state of things is easily demonstrated, since the rays of light that act as a stimulus can easily be directed to a single point. On the other hand, it can hardly be thought of as practicable to expose the root-tip alone to the stimulus of gravitation, or in place of this to centrifugal force. We attained the end in another way, however, namely by compelling the tip of a root, whilst growing quite normally, to permanently take up a position at right angles to the rest of the root.

For this purpose we allowed roots of *Faba*, *Lupinus*, &c., to grow into short tubes of thin glass that were bent at a right angle. The advancing root easily follows the bend of the tube and pushes on as far as the other end which has been closed by heat. Corresponding to the shape of the glass, there is now a terminal portion of the root, 1, 5 or 2 mm. long, at right angles to the rest of the root, of which again 1, 5 or 2 mm. occupies the other arm of the tube. This condition of things is continuously maintained, since, with its growing region in the tube, the older parts of the root, like plastic wax, are pushed out of the glass cap.

To prevent geotropic stimulus, the roots were made to

revolve slowly about their own axis on a klinostat, while they were growing into the tubes. If now a specimen thus prepared is placed so that the terminal part points vertically downwards, whilst the rest of the root is horizontal, no geotropic curvature takes place. This, however, always took place, and with about the same promptness as in straight roots, when the terminal portion was directed horizontally, or in general at an acute angle with the normal position.

From these experiments it follows that the root thus treated is perfectly capable of reaction. A geotropic reaction, however, only follows when the tip of the root is not placed in the position of equilibrium, that is when it is inclined from the vertical. But if the tip is directed vertically downwards, the rest of the root may occupy the horizontal or any other position, without any geotropic reaction following.

By this means therefore it is proved with the most perfect certainty, that in an uninjured root only the root-tip is geotropically sensitive.

On the Presence of Centrospheres in Fungi.

BY

HAROLD WAGER, F.L.S.

Lecturer on Botany in the Yorkshire College, Victoria University.

With Plate XVII.

THE presence of centrospheres appears to be of general occurrence in the cells of various groups of plants, as well as in animal cells. Our knowledge of them in plant-cells is chiefly due to the researches of Guignard [1], Bütschli [2], Strasburger [3], Schottländer [4], Lauterborn [5], and Farmer [6].

So far they have been found in plants chiefly in connexion with reproductive cells. They appear to have a very important function in the life of the cell. The karyokinetic division of the nucleus is directly under their influence, in that they appear to exert an attractive force upon the chromatic segments and cause their separation into two groups. They have been, in consequence, called 'kinetic centres,' and they ought to be found in connexion with all nuclei during division.

[1] Compt. Rend. Acad. des Sci. 1891; Ann. des Sci. Nat. Bot. 1891, Sér. 7, T. XIV.

[2] Reference in Strasburger's Verhalten des Pollens, &c., p. 57.

[3] Verhalten des Pollens, &c., Hist. Beitr. IV, 1892; Ueber die Wirkungssphäre der Kerne, &c., Hist. Beitr. V, 1893.

[4] Cohn, Beitr. z. Biol. d. Pflanzen, 1892.

[5] Reference in Strasburger's Wirkungssphäre, &c., p. 107.

[6] Annals of Botany, Vol. viii, No. XXX, 1894.

The observations on plant-cells which have been made up to the present time point to the existence of centrospheres as independent units of the cell originating in the cytoplasm, inasmuch as they are always found outside the nucleus and undergo division on their own account. In animal cells, on the other hand, they are often found to originate in the nucleus, and it is possible that future observation will show that even in plants the centrospheres are sometimes of nuclear origin. We will, however, refer to this question again later.

Centrospheres have not yet, so far as I am aware, been discovered in the cells of Fungi, if we except Gjurasin's observations on radiating striae at the poles of the nuclear spindle in an Ascomycete [1], and my own observations on the small centrosome-like body at the poles of the spindle in certain Hymenomycetes [2]. But recently, while working at the nuclei in the basidia of *Agaricus* (*Mycena*) *galericulatus*, in order to confirm the facts of nuclear division which had been discovered in other Hymenomycetes, I observed constantly a peculiar body (sometimes two) of somewhat large size, in connexion with the large nuclei of the basidium. This puzzled me for some time, and I at first thought it was merely an accidental occurrence due to some change produced by my method of preparation; but its constant appearance, regular size and definite staining properties led me at last to suspect that this body was a definite structure belonging to the cell and intimately connected with the nucleus. On making more careful observations I came to the conclusion that it was archoplasmic in nature and had to do with the formation of the centrosome-like body and spindle-figure, which are found in this fungus, and which had been already discovered in other Agarics. The following account of these structures will perhaps serve to elucidate somewhat their nature, and will give readers of this paper, who are interested in these bodies, an opportunity of judging whether my surmise

[1] Ber. der deut. bot. Gesell. 1893.
[2] Annals of Botany, Vol. vii. 1893, p. 489.

as to their archoplasmic nature is justified. I shall speak of them in this paper as archoplasmic bodies, simply for convenience, and not with any intention of definitely stating that they are such.

It is only by the most careful preparation and staining that these bodies can be seen at all. In cases where the sections have been but slightly overstained they have been visible only with great difficulty, and in badly stained specimens they could not be seen at all. The method used has been already referred to in a previous paper[1], and it will not be necessary to further allude to it here.

At present I have only been able to observe these archoplasmic bodies in connexion with the nuclei of *A. galericulatus*, but I have now and then observed with difficulty structures resembling them in the basidia of *A. stercorarius* and *A. mucidus*, but probably on account of the density of the protoplasm or of some imperfection in the method of preparation, I have not been able to see them clearly. The observations in this paper must be confined therefore to *A. galericulatus*. The process of nuclear division in the basidia of this species is identical with that already described in *A. stercorarius* and *A. muscarius*, with the exception of the appearance of the archoplasmic bodies.

If we examine a full-sized basidium, in which nuclear division has not yet commenced, we find that it contains a single large nucleus of elongate shape, placed about halfway up it, the long axis of which coincides with the long axis of the basidium. The remainder of the basidium is filled with granular protoplasm, which, fortunately, does not stain very deeply, so that the nucleus and the archoplasmic bodies are capable of being clearly differentiated. The nucleus possesses a somewhat indistinct nuclear membrane. Inside the nuclear membrane is a very distinct loose network in which the chromatin-granules can be distinctly seen, and a very large deeply stained nucleolus, generally placed on

[1] Loc. cit., Annals of Botany, Vol. vii.

one side of the nucleus. These different structures behave towards the double stain as follows:—The protoplasm is pale red with a bluish tinge; nucleolus deep blue-red; network also bluish red or blue.

Just outside the nucleus and generally in close contact with it are one or two bodies of somewhat large size, generally about the same size as the nucleolus when only one is present, a little smaller when two exist. They are coloured light blue and are easily overlooked, especially in badly stained or only slightly stained specimens. These bodies were always seen at this stage (see Figs. 8 and 9), in all those cases where the nuclei could be distinctly observed. Each appears to consist of a homogeneous mass of substance, spherical in outline. When two of these bodies occur, which is a preliminary stage to that when only one is present, they are not always in the same position. Sometimes both of them may be found on the upper side of the nucleus, or laterally, or they may occur one at each end of the nucleus, and generally they are in close contact with it; only occasionally are they removed a slight distance away. So far as the structure of these bodies is concerned, it differs considerably from that described by Guignard and others as existing in the centrospheres of plant-cells. There is no indication of a medullary corpuscle or centrosome. The bodies simply present the appearance of the so-called 'Nebenkern' of many observers, and answer more nearly to the description given by Hermann[1] of the archo-plasmic bodies in the spermatocytes of Salamander and Proteus, or in the cells of the embryonic genital ridge of the larval Salamander as described by Moore[2]. The latter observer points out that in these cells the archoplasm is a large pale grey mass, nearly as large as the nucleus itself; but when most conspicuous it is relatively small, and presents, in this condition, even with the highest powers, only doubtful suggestions of medullary zone or central body. We have

[1] Archiv für mikroskopische Anat. XXXVII.
[2] Quart. Jour. Microsc. Sci. 1893.

then an archoplasmic body, which answers very nearly to the description of these structures in *A. galericulatus*.

However irregularly these bodies may be placed at first, they gradually approach one another at the apex of the nucleus and fuse together into the single large body already mentioned, which presents the same appearance and reaction towards stains as the two original bodies. It is at first in close contact with the nucleus, and the nucleus still lies about halfway down the basidium (Fig. 9). At a later stage the archoplasmic body makes its way to the top of the basidium and becomes removed, therefore, to some distance from the nucleus (Fig. 10). Soon afterwards, however, the nucleus itself makes its way to the apex of the basidium and is again placed in close contact with the archoplasmic body. It looks as if the archoplasmic body exerted an attractive force upon the nucleus (Fig. 11). The nucleus is now in position for division, which always, without exception, takes place at the upper end of the basidium. While these changes have been taking place the nucleolus takes up a position at the base of the nucleus, so that we have now the archoplasmic body at the apex and the nucleolus at the base (Fig. 12). The nucleolus gradually loses its capacity for deep staining, and at the same time the nuclear threads become more and more deeply stained and are gradually removed to the apex of the nucleus, as already described in my previous paper[1] (Fig. 13). The threads become gradually divided up into the chromatic elements, which stain a deep bright red, the nucleolus becomes fainter and fainter in colour, the nuclear membrane gradually disappears and the remains of the nucleolus are extruded into the protoplasm, where they often divide up into two or more small spherical bodies (Fig. 16) and finally these completely disappear.

While the chromatic elements are being thus developed, the centrosphere undergoes a change. At first it is seen in close contact with the nuclear threads, as they accumulate at the

[1] Loc cit.

apex of the nucleus, as a light blue homogeneous mass more or less spherical in outline. This gradually becomes more and more irregular until it can only with difficulty be distinguished from the surrounding protoplasm, and finally it also disappears (Figs. 13 and 14).

Shortly after this the spindle-figure appears with the two centrosomes, one at each end. It is difficult to understand exactly how this takes place. Hermann's description of the process as it occurs in the Salamander and Proteus is very suggestive[1]. He points out that in the archoplasmic mass at first no centrosome could be seen, but when the nucleus gets to the stage when the threads begin to split longitudinally, the structure of the archoplasm becomes clearer, and one can then distinctly make out two centrosomes which repel one another, and between them is the young spindle. As the spindle increases in size, threads radiate out to the chromatic elements and come into contact with them. The spindle and radiating threads are thus gradually formed out of the archoplasmic body.

Lauterborn[2] has described a very interesting case of spindle-formation in a Diatom. The structure which corresponds to the nuclear spindle proceeds from a body which exists beside the centrosome. This body however, according to the author, might arise from the centrosome, and he proposes to compare it with the 'Nebenkern' of animals.

Something of the kind described by Hermann may take place in *A. galericulatus*. The spindle is probably formed out of the substance of the archoplasmic body as this becomes invisible. The centrosomes appear first, and from these start the radiating threads, some of which form a spindle, and some come into contact with the chromatic elements (Figs. 15 and 16). The chromatic elements then take up their position at the equator of the spindle to form the equatorial plate. The centrosomes at this stage are very clearly defined as small spherical bodies, stained blue. The spindle-threads are also stained blue. The centrosomes remain visible during the

[1] Loc. cit. [2] Loc. cit.

process of separation of the threads, until the groups of chromatic elements begin to fuse together at the poles (Fig. 20); after that they become invisible. I could not determine whether they disappear altogether or not (Fig. 21).

The two daughter-nuclei are gradually formed as described in my previous paper, but I could not at any stage detect anything of the nature of an archoplasmic body in contact with them. This might be due to the difficulties in the way of preparation or staining. At a later stage, when the spindles of the daughter-nuclei appear, two centrosomes can be made out at the poles of the spindle, but these are not quite so clear as in the original parent-nucleus. The four daughter-nuclei produced, gradually expand until they become similar to the parent-nucleus. The various stages are figured in Figs. 23–29, but at no stage of their development could anything of the nature of an archoplasmic body (as distinguished from the centrosomes) be made out. This, however, is not equivalent to saying that archoplasmic bodies or centrospheres are not present. It may be that owing to imperfections in the method of preparation they were not rendered visible, or some change may have taken place of which we are not cognizant, owing to our scanty knowledge of archoplasmic structures and their nature. We cannot therefore at present state what is the ultimate fate of the archoplasmic body in *A. galericulatus*, and we are in much the same difficulty as regards its origin. In fact the whole question of the origin of the centrospheres, whether they are derived from the cytoplasm or from the nucleus, cannot yet be satisfactorily answered. Some observers incline to one view, some to the other. It certainly appears as if, in the majority of cases, the centrospheres were always outside the nucleus, and Guignard's observations favour this view for plant-cells [1], but in animal cells there are cases where the centrosphere appears to be distinctly nuclear in origin. August Brauer [2] describes the centrospheres in the spermacyte

[1] Loc. cit.

[2] Archiv f. Mik. Anat. 1893, pp. 285–7; Bio. Cent. 1893, p. 197.

of *Ascaris megalocephala*, var. *univalens* as being nuclear in origin. His figures are certainly very clear and show the centrospheres distinctly inside the nucleus. The division of the centrospheres into two and the formation of the spindle-figure may all take place within the nucleus, but these bodies are finally extruded through gaps in the nuclear membrane into the cytoplasm, where they become surrounded by radiating striae. In some cases, even, the centrospheres may pass to the outside before dividing. The author regards both centrospheres and spindle-figure as nuclear in origin.

O. Hertwig[1] also states that the centrospheres belong to the resting nucleus and only come out into the cytoplasm for division, and it is only in special cases that the centrosphere remains in the cytoplasm during the resting stage of the nucleus.

Hausemann[2], from observation on cells in pathological tissues, also supports this view.

Strasburger[3] summarises the various cases of centrosphere- and spindle-formation somewhat as follows:—

(1) The centrosphere is found inside the nucleus, and the whole of the nuclear spindle is formed from the contents of the nucleus.

(2) The centrosphere lies outside the nucleus, but the spindle owes its origin to a substance which is formed in the nucleus.

(3) The spindle is formed partly from a substance in the cytoplasm, and partly from a substance in the nucleus.

(4) The spindle is formed out of a substance which only occurs in the cytoplasm. This is universal for plant-cells.

Moore[4] has recently shown that in the reproductive elements of *Apus* and *Brachipus* the centrospheres are derived from a massing together of a number of deeply stained bodies, which he calls pseudosomes, found in the angular spaces in a network of protoplasm exterior to the nucleus; and Farmer[5]

[1] Die Zelle und die Gewebe, 1893. [2] Anat. Anzeiger, VIII, 1892.
[3] Ueber die Wirkungssphäre, &c., Hist. Beitr. V, 1893.
[4] Q. J. M. S., 1893. [5] Annals of Botany, Vol. VII, 1893.

gives a somewhat similar account of the process in the pollen-mother-cells of *Lilium Martagon*, the same species which was investigated by Guignard, with a totally different result.

Zimmermann[1] has also shown that, during karyokinesis, bodies appear in the protoplasm which correspond with nucleoli in their behaviour towards certain stains. He regards these bodies as arising directly through the breaking up of the nucleolus. Whether these are to be regarded as similar to those described by Moore and Farmer is doubtful. However, all these observations seem to show that in some cases, the centrospheres have a nucleolar origin[2]. But so far as the observation, already referred to, of Guignard and others on plant-cells are concerned, the centrospheres appear to have a perfectly independent origin apart from the nucleus. According to Guignard all centrospheres arise by the division of pre-existing centrospheres, and these always lie outside the nucleus. My own observations on *A. galericulatus* seem to me to support the nuclear and even nucleolar origin of the centrosphere. I have carefully examined nuclei in all parts of the Fungus, both hyphae and basidia, and I have been able to follow more or less completely the gradual building up of the large nucleus of the basidium, and have been also able to determine the stage at which the archoplasmic body appears. The nuclei of the hyphae are quite small and stain deep red (Fig. 1). Each nucleus consists of a nuclear membrane enclosing a few granules or threads; but no nucleolus is to be seen. And I have not been able, even with the most careful staining, to discover anything of the nature of an archoplasmic body in contact with the nucleus at this stage. They would not be easily overlooked unless they were very minute, as the protoplasm occurs in very small quantities in the hyphae and does not stain at all deeply. In fact it is quite easy to get

[1] Morph. und Physiologie d. Pflanzenzelle. Band II, Heft 1.

[2] The subject has quite recently been re-investigated by J. E. Humphrey, who criticizes the statements of Zimmermann, Farmer, &c., and comes to the conclusion that nucleoli and centrosomes have no connexion with each other; Ber. d. deutsch. bot. Gesellschaft, 1894, Heft 5: see also his note in the present number of the Annals.

the nuclei clearly stained whilst the protoplasm remains unstained. These nuclei pass into the basidia, but at first the basidia are quite destitute of nuclei and contain two or three vacuoles (Fig. 2). The presence of vacuoles, as well as the size of the basidium, afford good indications of its age, so that it is quite easy, in looking over large numbers of sections, to arrive at a perfectly safe estimate as to the stage which the basidium has reached in its development. By working in this way I have been able to make out certain facts which indicate, as before mentioned, the nuclear origin of the centrospheres.

The quite young basidium does not contain nuclei (Fig. 2), but it does not remain long in this condition. Very soon after its formation nuclei pass into it from the hypha, upon which it is borne. The number of nuclei which pass in at first is two, and I have never seen a basidium with more than two nuclei at this stage (Fig. 3), but more than two pass in ultimately. These nuclei are precisely similar in structure to those found ordinarily in the hyphae, except that they may be a little larger, and a trifle more distinct. The chromatic elements inside them stain deep bright red, so that they are easily seen. In basidia, at a slightly later stage of development, the two nuclei are found to have increased in size, and a nucleolus just begins to be visible. The chromatic elements still stain bright red, but the nucleolus stains somewhat blue and is very indistinct. As the basidia develop, these nuclei become larger and the network becomes more distinctly visible; the nucleolus also increases in size and can now be very easily seen. At this stage the nucleolus stains bluish red, and the chromatin-network is still red, but the colour is not so bright as before, and there is perhaps a tinge of blue to be made out in it. As the nucleus becomes older the chromatin-network tends to become more blue, and the nucleolus tends to take up more of the red stain (Fig. 4).

It is interesting to compare these developmental changes with those which take place later in the gradual formation of the daughter-nuclei, after division, in the basidium, as they are almost precisely similar, and give indications of one of

the functions of the nucleolus, viz. that of storing up the substance which causes the chromatic elements to stain red. The structure of the four daughter-nuclei in the basidium, just at the moment of their formation or shortly afterwards, is precisely similar to that of the hyphal nuclei. They possess a few chromatic elements stained red, but no nucleolus. As they increase in size, however, a nucleolus appears, and gradually the bright red colouring of the chromatic thread disappears and is replaced by the light or dark blue coloration which is found in the normal basidial nuclei. It appears as if in both cases the advent of the nucleolus caused the substance which produces the bright red staining of the threads to be given up, and if we regard the nucleolus as a storehouse of this substance, as appears probable from previous observations, we can, I think, understand more clearly the gradual formation of the nucleoli in the young primary nuclei of the basidium.

The increase in size of these primary nuclei is probably due partly to growth, and partly to the fusion of nuclei. The nuclei appear to fuse together in pairs, but I have not been able to follow this out satisfactorily. If such fusion does take place, it probably occurs both in the basidium and in the hyphae. But by whatever means the nuclei increase in size, a stage is at last reached when we have in the basidium two nuclei, each with a distinct reddish network and a single well-defined nucleolus which takes a reddish blue stain. No kind of archoplasmic body is visible at this stage. These two nuclei fuse together. I have seen them fusing, but have not been able to follow out all the details. At the same time similar nuclei pass into the basidium from the hypha and they also fuse together rapidly. The basidium now contains two nuclei, produced by the fusion of four pre-existing nuclei, and in contact with each nucleus, in some cases looking as if it were just being extruded from it, we can see a spherical body precisely similar to the nucleolus and stained in a similar way (Fig. 5). It looks exactly as if it were a nucleolus, the second one being extruded from the fused nucleus, and the fact that

this body as well as the nucleolus left in each nucleus are precisely similar in size, so far as could be made out by observations on different basidia, to the nucleoli of the original nuclei before their fusion, supports this view. These are the archoplasmic bodies. They become completely separated from the nuclei, and in some cases removed to some little distance from them. We have now in the basidium two nuclei, each with one of these archoplasmic bodies in contact with it. Sometimes they are placed in front of each nucleus, sometimes behind. They gradually lose their red colour and stain only blue (Fig. 6).

The two nuclei now fuse together to form one large nucleus (Figs. 7 and 7 *a*). The archoplasmic bodies remain separated for some time, but finally undergo fusion as described in a previous part of this paper. The nucleoli remain for a short time separate from one another, but they soon fuse together also, and we have then in the basidium a single large nucleus, to which is attached the archoplasmic body.

EXPLANATION OF FIGURES IN PLATE XVII.

Illustrating Mr. Wager's paper on Centrospheres in Fungi.

C. Archoplasmic body (Centrosphere).
Cs. Centrosome.
N. Nucleolus or remains of nucleolus.

All the figures have been drawn with the help of the Camera lucida and the apochromatic, 2·0 mm., 1·4 apert., object-glass of Zeiss and ocular 18.

Fig. 1. Piece of hypha from a gill, with two nuclei; no nucleoli visible; the chromatin coloured red.

Fig. 2. Young basidium without nuclei; numerous vacuoles present.

Fig. 3. Young basidium with two nuclei, which have probably just passed in from the hyphae. The structure of the nuclei similar to those in Fig. 1, but the nucleoli are just visible as small, faintly stained bodies.

Fig. 4. Basidium with two larger nuclei; chromatin coloured bright red; nucleoli distinctly visible and coloured dark reddish purple. Each of these two nuclei has probably been formed by fusion of nuclei similar to those in Fig. 3.

Fig. 5. Basidium with two nuclei separated from each other by a vacuole. Nuclei somewhat larger than in Fig. 4. The archoplasmic bodies, *c*, are now visible for the first time and are coloured faint blue. They are in close contact with the nuclei, and look almost as if they were being extruded from them. These nuclei have probably arisen by fusion of such nuclei as in Fig 4; the chromatin-network is contracted away from wall of nucleus just as it is in cases of fusion of older nuclei.

Fig. 6. Young basidium with two nuclei, later stage. Chromatin-network very dense, coloured red. Nucleolus dark red. Near each nucleus, but distinctly separated from it, is the archoplasmic body, which stains light blue.

Fig. 7. Basidium with two nuclei just beginning to fuse together; the granular network clearly visible. Two archoplasmic bodies on opposite sides of the fusing nuclei. Nucleoli bluish red, network also bluish red, archoplasmic bodies faint blue.

Fig. 7 *a*. Basidium with nuclei just after fusion. The two nucleoli still distinctly separated from one another. Archoplasmic bodies at opposite sides of the nucleus.

Fig. 8. Much later stage. Nucleus much elongated. Nucleus threads now distinct with chromatin-granules. Nucleolus large; archoplasmic bodies close together at apex of nucleus. The nuclear network appears to be breaking up into separate equal sized threads.

Fig. 9. Slightly later stage. Nucleolus at extreme apex of nucleus. Archoplasmic bodies have fused together and now appear as a slightly elongate light blue body.

Fig. 10. Archoplasmic body at some distance from apex of nucleus which is still in same position, about halfway between apex and base of the basidium, as in Figs. 8 and 9. Nucleolus still at extreme apex of nucleus.

Fig. 11. Basidium with nucleus nearer its apex. Archoplasmic body in close contact with the nucleus in upper part. Nucleolus now halfway down.

Fig. 12. Archoplasmic body at apex of nucleus. Nucleolus now at opposite end.

Fig. 13. Later stage. Chromosomes, condensing at apex of nucleus, deep bright red in colour. Near these lies the archoplasmic body, somewhat irregular in shape. At the base two bodies in close contact with each other, light blue in colour. These are due to division of nucleolus, which sometimes takes place at this stage. The nucleolus loses its colour also at this stage.

Fig. 14. Nearly same stage as Fig. 13, or perhaps little later. Archoplasmic body is not now visible.

Fig. 15. Transverse section of basidium, showing a few chromosomes and the centrosomes from which radiating striae are beginning to pass. The centrosomes are stained light blue. This is a stage just before spindle-figure becomes clearly visible.

Fig. 16. Longitudinal section of basidium, cut somewhat obliquely to the plane in which centrosomes are placed, so that only one of these is visible.

Some of the striae radiating from the centrosome are in contact with the dense mass of chromosomes. Two small bodies (remains of nucleolus) below the group of chromosomes.

Fig. 17. Longitudinal section of basidium also cut so as to show only one centrosome. Threads of spindle distinctly seen. Some radiations from centrosome into protoplasm only. Remains of nucleolus below.

Fig. 18. Basidium with spindle-figure and centrosomes distinct. Spindle-threads and centrosomes light blue in colour, very distinct; equatorial plate of chromosomes bright deep red.

Fig. 19. Transverse section of basidium. Chromosomes beginning to separate into two groups. Spindle-figure and centrosomes clearly seen.

Fig. 19 *a*. Same stage as above, but in longitudinal section. Centrosomes very distinct. Remnant of nucleolus just visible some distance below.

Fig. 20. Later stage. Chromosomes in two groups near ends of spindle. Centrosomes still just visible as faintly-coloured light blue bodies.

Fig. 21. The two groups of chromosomes just before fusing to form the two daughter-nuclei. The centrosomes are not visible.

Fig. 22. Chromosomes fused to form the two daughter-nuclei. Each appears to be perfectly homogeneous and stained bright red.

Fig. 23. The two daughter-nuclei just expanding. Network just beginning to be visible, stained bright red, and numerous deeply stained granules.

Fig. 24. Two daughter-nuclei completely formed; network and nucleolus distinctly visible; no archoplasmic bodies visible.

Fig. 25. Transverse section of a basidium, showing two daughter-nuclei dividing. Centrosomes very small.

Fig. 26. Longitudinal section of basidium, in same stage as Fig. 25.

Fig. 27. Four daughter-nuclei, homogeneous.

Fig. 28. Daughter-nuclei expanding; network begins to be visible.

Fig. 29. Network of daughter-nuclei distinctly visible; several deep red granules to be seen. At a later stage the nuclei are similar to those in Fig. 24, but slightly smaller.

New Marine Algae.

BY

E. M. HOLMES, F.L.S.

With Plate XVIII.

THE careful exploration of the shores of Natal, and especially of the mouth of the Kowie River, by Dr. Hermann Becker, has led to the discovery of many interesting marine Algae, and of the fructification of some imperfectly known species. Some of those sent have already been described [1].

I now propose to describe a few more species that Dr. Becker has recently sent for examination. One of the most interesting of these plants is a species which was published as long ago as 1842 by Hering under the name of *Gelidium aculeatum*, but which has never, until now, been found with cystocarpic fruit. It bears so singular a resemblance in form and habit to *Eucheuma spinosum*, J. Ag., that it was sent by Dr. Becker under that name. Agardh has recently (before he had seen cystocarpic fruit) referred it provisionally to the genus *Coral-*

[1] *Enteromorpha rhacodes*, Holmes, Grevillea, vol. 22 (1894), p. 89; *Phacelocarpus epipolaeus*, Holmes, Trans. Bot. Soc. Edinb., 1894; *Tyleiophora Beckeri*, J. Ag., Till. Alg. Syst. VI, p. 36; *Holmesia capensis*, Till. Alg. Syst. Pt. VII, T. 1, Fig. 4, p. 39.

[Annals of Botany, Vol. VIII. No. XXXI. September, 1894.]

lopsis. We are now able to confirm this opinion, having examined the cystocarps. It may now therefore be described as follows.

CORALLOPSIS ACULEATA, nobis.

Syn. *Gelidium aculeatum*, in Ann. and Mag. Nat. Hist. (1842), no. 91; Krauss, Regensburg Flora (1846), p. 210; J. Ag., Sp. Alg. p. 629.

Sphaerococcus Heringii, Kütz., Sp. Alg., p. 775.

Gigartina aculeata, Kütz., Tab. Phyc. vol. xvii. tab. vi.

Descr.:—*Fronde usque pedali, cartilagineo-cornea, 1–2 lin. crassa, inferne caulescente teretiuscula, mox dichotomo-corymbosa, ramis basi eximie constrictis, 4–5 angularibus, aculeatis, aculeis oppositis, vel ternatim, quaternatim verticillatis, lineam longis basi dilatatis, horizontalibus, interstitiis verticillorum bilineariibus; colore coccineo-purpureo; coccidiis ad ramos superiores numerosis; sphaerosporis cruciatim divisis in ramis ultimis crassiusculis immersis.*

The singular resemblance that this plant bears to *Euchcuma spinosum* apparently led Kützing to refer it to the genus *Gigartina*, under which *E. spinosum* had up to that time been placed. Agardh also, whilst provisionally retaining Hering's original name of *Gelidium aculeatum*, placed it (in 1852), as an undetermined plant, under the genus *Eucheuma*, remarking, however, that although the habit was that of *Eucheuma*, the structure was quite different and resembled that of *Gracilaria*, except in the cells of the cortical stratum forming very short filaments, a point in which the more recent genus *Corallopsis* differs from *Gracilaria*. The other prominent feature in which *Corallopsis* differs from this genus is in the more or less subarticulate appearance of the fronds, which is not well brought out in Kützing's illustration of *Gigartina aculeata*. In this species it is chiefly confined to the base of the branches, which are much narrowed, particularly in the tetraspore-bearing plant. It is more nearly allied to *Corallopsis Urvillei*, J. Ag. than to the other species of the genus, from which it differs in the verticillate spines and less branched frond.

PTILOTA CRYPTOCARPA, n. sp.

Fronde ad duas pollices longa, decomposito-pinnata; pinnis rachide compresso-plana, alternis, bipinnatis patentibus; pinnellis a latiore basi acuminatis, terminalibus forcipatis, sterilibus integris, fertilibus apicem versus phyllis articulatis callithamnioideis plurimis vestitis; favellis in ultimas pinnellas terminalibus, filis articulatis callithamnioideis dense circumdatis occultisque; sphaerosporis tripartitis, inter filamenta callithamnioidea brevissima nidulantibus, ad pinnellarum apicem sparsis; colore atro-purpureo.

Hab. Near the mouth of the Kowie River, parasitical on the stem of *Phacelocarpus tortuosus.*

This species belongs to a group of the Ptilotae, which is very distinct in facies from the rest of the genus, and indeed bears some resemblance to *Phacelocarpus* in habit. The other species of this group are *Ptilota rhodocallis*, Harv., *P. siliculosa*, Harv., and *P. striata*, Harv. From all of these, however, it differs in its dwarf stature, in the strongly forcipate tips of the sterile pinnae, the velvety coat of involucral filaments surrounding the favellae and extending some distance below them, and in the terminal position of the favellae. In the tetraspore-bearing plants the callithamnioid filaments are visible, only under a lens.

Professor Schmitz has also received the plant I have named *Ptilota cryptocarpa*, and has referred it in MS. to *Dasyphila* under the name of *D. minor*. It seems doubtful to me, however, whether these two genera should not be united, the section of *Ptilota*, to which *P. striata* belongs, being closely allied to *Dasyphila*. Indeed, Harvey (Phyc. Aust. II, Pl. LXVI) remarks under *Dasyphila Preissii*: 'This handsome plant might, without much violence, be considered as a species of *Ptilota*, from which genus *Dasyphila* differs merely by having the frond covered with a velvety stratum of microscopic filaments. There is no essential difference in the fructification, especially if we compare it with our *Ptilota striata*, Pl. LXXII, which may almost be regarded as a glabrous *Dasyphila*, if such

were admissible.' The present species forms a still closer connecting link between the two genera, since the incurved callithamnioid filaments, which cover the frond in *Dasyphila*, are confined to the tips bearing favellae and tetraspores in *P. cryptocarpa*, the rest of the frond resembling *Ptilota striata*, in which species they are strictly confined to the favellae. The chief difference between the two genera appears to be in the terminal position of the favellae on the branchlets and pinnules, and in this feature *P. cryptocarpa* certainly more nearly approaches *Dasyphila*. We have not, however, been able to observe in the favellae a 'placentula' from which the spores radiate, and which is said to be present in *Dasyphila*. The favellae appear in the specimens I have examined to be 2 or 4-lobed, each lobe surrounded by a hyaline periderm. On the whole, therefore, it seems to me preferable to place this remarkable plant in *Ptilota*.

GLAPHYRYMENIA PORPHYROIDEA, Schmitz, MS. n. sp.

Fronde late expansa gelatinoso-membranacea, opaca, oblongo-rotundata, margine, undulata; colore roseo-purpureo; favellis sparsis in strato medio immersis.

The structure of the frond and of the favellae indicates that this plant belongs to the genus *Glaphyrymenia*. The plant, in the single specimen received, is somewhat injured, so that it is difficult to judge of the size or shape of the entire plant. The frond appears to consist of two or three broad lobes of an oblong or rounded-oblong form, about five inches long by three broad. The surface is not glossy as is usual in *Porphyra*, but dull and lustreless. The substance, when wetted, is seen to be soft and gelatinous, although very thin. The colour is of a peculiar pale purplish rose tint.

Hab. 'Cape of Good Hope,' Dr. H. Becker.

I had from imperfect sections concluded that this plant belonged to the genus *Halymenia*, but Professor Schmitz has pointed out that the cystocarp presents more nearly the structure of *Glaphyrymenia*.

MICROCOELIA KALLYMENIOIDES, n. sp.

Fronde breviter stipitata late expansa gelatino-carnosa, obliquo-lanceolata vel reniformi, ad 12 poll. longa, 3–4 poll. lata, margine integro-subincrassata; sphaerosporis tripartite divisis, in strato corticali immersis, per totam superficiem sparsis; colore coccineo-purpureo.

Hab. Near the mouth of the Kowie River, 1883, Dr. H. Becker. This genus is as yet only represented by a single species from Chili (*Microcoelia chilensis*).

Externally, the African species bears a singular resemblance to *Kallymenia reniformis*, and but for its dull brownish red tint, might almost be taken for an old specimen of that plant. *Microcoelia*, however, differ from the genus *Kallymenia*, as pointed out by Agardh, in the presence of a series of gradually smaller horizontally placed cells between the large central cells of the frond and the cortical layer. From *Callophyllis*, which the structure somewhat resembles, it differs in the very gelatinous and badly defined walls of the larger cells, and from both genera in the tripartite tetraspores, which have not been found in *M. chilensis*.

The nearly sessile fronds arise in a cluster of three or four from the base, which is imperfect. On the margin of the frond where it is injured, small rounded obovate short-stalked proliferations are formed, which indicate that the adult plant also possesses a short stem. In outline the young basal fronds are lanceolate, but the older ones are more reniform than lanceolate, one side of the frond being much more developed than the other. The internal structure of the frond agrees well with that of *Microcoelia*, as described by Agardh. In the centre of the frond, there are large cells parallel to the surface, with gelatinous badly defined walls, followed by two or three rows of gradually smaller cells, the cortical layer being thin and composed of minute cells vertical to the surface. The tetraspores are apparently formed from the cortical cells (which alone are coloured), but occupy cavities formed partly in the cortical, and partly in the sub-

cortical layer. Professor Schmitz is of opinion that the plant belongs to the genus *Hymenocladia*, and has given it the MS. name of *H. kowiensis*. He states that he has seen the cystocarpic plant, but the specimen that he alludes to is not, I think, identical with *M. kallymenioides*.

PACHYMENIA RUGOSA, n. sp.

Fronde cartilagineo-carnosa, callo radicali crasso conico brevi oriunte, late expansa, undulata vel plicata, aetate corrugata; colore coccineo-purpureo. Favellae infra stratum corticale immersis, per frondem sparsis.

Hab. Near the mouth of the Kowie River, Dr. H. Becker.

The specimens received have a more or less denticulate or eroded margin, which appears to have been caused by mollusks, since in places the margin is entire, and where it is not so it has become double with a furrow between the two marginal lines. As in other species of this genus the nucleus of the cystocarp is surrounded by a flask-like arrangement of filaments, the mouth of the flask projecting slightly above the surface of the frond and thus forming the carpostome. The wrinkled character of the frond does not however appear to be due to the presence of numerous carpostomata, but to irregular thickening of the tissues. The small cells, vertically arranged, forming the cortical layer, usually consist of about nine rows of moniliform coloured cells, those nearest the surface consisting of about three rows of minute cells. In the rugose portion this superficial layer is thicker. Professor Schmitz proposes to place this plant, which he identifies with *Iridea cornea*, Kütz., Tab. Phyc. xvii. 23, in a new genus *Cyrtymenia*, together with *Grateloupia hieroglyphica*.

MYRIOPHYLLA, nov. gen.

Frons gelatinoso-carnosa, cylindraceo-compressa, stratis duobus contexta, interiore cellulis magnis oblongis pluriseriatis, cellulis minoribus interstitiis replentibus, superficiem versus cellulis gradatim minoribus strato corticali tenui, cellulis minutis constituente. Sphaerosporae in phyllis minutis lanceo-

latis obtusis totam frondem supra basim dense distiche obtegentibus, cruciatim divisae, in strato corticale immersae.

MYRIOPHYLLA BECKERIANA, n sp.

Fronde bipedali longa, ad duas lineas lata cylindraceo-compressa, irregulariter et distanter dichotoma apice attenuata, phyllis sphaerosporiferis per totam longitudinem ramorum distiche vestitis.

Hab. Near the mouth of the Kowie River, Dr. H. Becker.

This remarkable plant has a habit peculiarly its own. From the beginning of the branching of the frond it is densely fringed on the two sides with minute leaflets, bearing tetraspores. These are not, as in *Delesseria*, borne on a denuded midrib, but at the edge of the frond as in *Carpoblepharis*, and are filled with a delicate tissue of large thin-walled cells. The cystocarpic fruit is hitherto unknown, and the exact position the genus should occupy cannot therefore be at present ascertained. In structure the frond approaches the species of *Chrysymenia*, in which the frond is solid, and the plant may provisionally be placed in the Rhodymeniaceae between *Chrysymenia* and *Cordylecladia*.

Since writing the above description we have heard from Professor Schmitz of Greifswald, that he has also received the same plant from Dr. Becker, with the cystocarpic fructification upon it, and he is of opinion that it should be referred to *Chrysymenia* and to the section *Botryoclada*, to which belong *C. uvaria* and *C. obovata*. On the other hand we have the opinion of Professor Agardh, who has, however, examined the tetraspore-bearing plant only, that the plant, although allied to *Chrysymenia*, should form a separate genus. Certainly the structure and consistence of the frond and the shape of the tetraspores are different from those of the *Botryoclada* section, and the leaflets bearing the fructification are not arranged in a spiral manner, but distichously, so that the plant bears a singular resemblance in habit to *Suhria vittata*, although quite different in consistence, which is that of the *Cryptarachne* group of *Chrysymenia*. In *Myriophylla* the large cells are

more angular, thinner-walled, and not rounded as in *C. uvaria*, and there are definite series of smaller cells inserted between the larger ones, the superficial layer of cells is almost monostromatic, the leaflets can scarcely be said to be inflated, being filled with thin-walled cells, and the tetraspores are spherical, not oblong, as in *C. uvaria*. If placed in the genus *Chrysymenia* at all, it should form a distinct group between the *Cryptarachne* and *Botryoclada* sections. We have not been able to examine the cystocarps, but Professor Schmitz's cystocarpic plant, of which he has kindly forwarded a sterile fragment, appears to be identical in structure with our plant.

EXPLANATION OF FIGURES IN PLATE XVIII.

Illustrating Mr. Holmes' paper on New Marine Algae.

MYRIOGLOSSA BECKERIANA, n. sp.

Fig. 1. Frond, half the natural size.

Fig. 2. Leaflet, bearing cruciate tetraspores.

Fig. 3. Longitudinal section of ditto.

Fig. 4. Tetraspores.

Fig. 5. Transverse section of frond, showing distichous arrangement of leaflets.

Fig. 6. Longitudinal section of frond.

Fig. 7. Transverse section of ditto.

PTILOTA CRYPTOCARPA, n. sp.

Fig. 8. Frond, natural size.

Fig. 9. Transverse section of stem.

Fig. 10. Longitudinal ditto.

Fig. 11. Longitudinal section of pinnule.

Fig. 12. Apex of pinnule, showing concealed favellae.

Fig. 13. Ditto in longitudinal section.

Fig. 14. Transverse section below favellae, showing involucrate ramelli.

Fig. 15. Apex of tetrasporic pinnule, showing forcipate ramuli clothed with short callithamnioid filaments.

CORALLOPSIS ACULEATA, J. Ag., MS.

Fig. 16. Ramulus bearing cystocarps, natural size.

Fig. 17. Cystocarp, (× 50) in longitudinal section.

Fig. 18. Ramulus bearing tetraspores.

Fig. 19. Section of ditto, showing cruciate tetraspores *in situ* (× 75).

Fig. 20. Tetraspores (× 200).

A Theory of the Strobilus in Archegoniate Plants[1].

BY

F. O. BOWER, D.Sc., F.R.S.

Regius Professor of Botany in the University of Glasgow.

IN previous communications to the Royal Society, to the British Association, and to the Annals of Botany, I have taken opportunity to state in part the views to which I have been led by the study of spore-producing members. Till now, however, I have not attempted to put into a comprehensive and connected form the chain of ideas which observation and comparison have suggested. I shall endeavour to state it now with all possible brevity.

I assume, for the purposes of this argument, that Hofmeister's general conclusions will be accepted: that antithetic alternation was constant throughout the evolution of archegoniate plants, and that the sporophyte has been the result of elaboration of the zygote. There is one further point on which a clear understanding is necessary from the outset of the discussion: in all those plants which show antithetic alternation, with the exception of a few aposporous forms which I regard as abnormal, spore-production is a constantly recurring event. A comparison of the Confervoideae and of the simpler Bryophyta shows that spore-production was the first office of the sporophyte, and in the simplest types it is seen to

[1] Read before Section D of the British Association at Oxford, August 1894.

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take place where vegetative tissues are practically absent. On comparative grounds we are therefore justified in concluding that the vegetative phase of the sporophyte has, in the course of evolution, been intercalated between fertilization and spore-production; accordingly, in point of evolutionary history, we should designate spore-bearing tissues of the sporophyte as *primary*, while vegetative tissues of the sporophyte would be regarded as *secondary*.

It will be our main object, by comparative study, to obtain a connected idea of some of the methods of progress of the sporophyte from its simpler to its more complex types. And here it must be expressly stated that the primary intention is not to trace actual homologies, or to demonstrate the phylogenetic relationships of known families of plants; we shall rather endeavour to arrive, on grounds of detailed examination and comparison, together with consideration of the biological position of the organisms themselves, at some reasonable view of the *methods of advance* of archegoniate plants.

We see on comparison of the simpler types, before any complications of external form, evidence of two chief factors of progress: viz. (1) *increase of spore-production*, and (2) *sterilization of some of the potentially sporogenous cells*. It will be shown that these two factors make their effects apparent among the more complex as well as among simpler forms. Taking first the increase of spore-production, let us consider it from the biological aspect: the advantage which it brings appears to be that by this means one sexual process is sufficient to produce a more numerous progeny; in archegoniate plants we are dealing with organisms probably derived from some aquatic Algal ancestors in which the zygote germinated directly, or after few divisions, to form new sexual plants; but the typical Archegoniatae are land-plants; they still depend, however, upon external fluid water for the movement of their spermatozoids, and thus fertilization is only possible at intervals when fluid water is present. I believe that we see in the extensive subdivision of the

zygote and formation of numerous spores, the way in which archegoniate plants evaded the difficulty which their land-habit presented to constantly recurrent fertilization; instead of many zygotes leading by direct germination to a supply of new individuals, the increase in number of the Archegoniatae was brought about by subdivision of the zygote, and formation of numerous carpospores. It would further appear that—other things being equal—the larger the output of these spores, the better the chance of survival of the species which produced them: a comparison of types of the homosporous Archegoniatae may illustrate a probable line of increase in spore-production: from the simple *Riccia*, through various larger Bryophytic forms, we may rise, with an ever-increasing output of spores, to such plants as the Equiseta, Lycopods, and Ferns, in which the output of spores was, and is, among the largest in the vegetable kingdom. I have calculated that a large Male Fern (by no means large among Ferns) may produce many millions of spores in each year; I leave it to others to estimate the number of spores produced annually by a large *Alsophila* or *Cyathea*; clearly the number is much larger than is matured from a single sporogonium of any Bryophyte, and it is to be remembered that while sporogonia are developed within one season, most homosporous Vascular Cryptogams are perennials.

In the heterosporous forms, however, with the sexual differentiation of the spores, there followed a reduction in the number of the female spores, counterbalanced by the greater certainty of success of the offspring, as following on the larger supply of nourishment contained in the enlarged female spores. The male spores still continued numerous, as in the case of Coniferous pollen; but finally the amount of pollen required to ensure fertilization was economized among those higher plants which show perfection in pollinating mechanism. Thus we may conclude broadly that the climax of numerical spore-production was among the homosporous Vascular Cryptogams.

But such an increased production of spores as we have

been contemplating could not be carried out without special means of nourishment of the spores during their development, and some mechanism for their dissemination when mature. On ascending from the simplest Bryophytes to the more complex Ferns, Lycopods, and Equiseta we see various devices for meeting these and other difficulties: these devices are more or less directly connected with the second factor, viz. *sterilization of some of the potentially sporogenous cells*; it will thus be seen that these two factors of progress have worked side by side, increase of spore-production bringing in its train the necessity of a partial sterilization. Frequently the results of the one factor are complicated by the occurrence of the other; while, further, the appearance of continued apical or intercalary growth may make it difficult, or even impossible, to form a definite opinion as to the part which the two factors have individually taken in the evolution of the organisms as we now see them.

Our next step will be to consider examples of the evidence that *sterilization of potentially sporogenous cells* has occurred; to recognize the various ends which the sterile cells serve, and to note how widespread the examples of sterilization are from the lower archegoniate plants upwards to the Angiosperms.

Starting with the simplest Bryophyta, it may be seen that differentiation of the cells of the sporophyte, though absent from their Algal prototypes, is always present in the Archegoniatae. In *Riccia* a peripheral layer of cells forms the protective wall of the sporophyte; from the lowest of archegoniate sporophytes upwards such protective tissues of the wall surround the spores, and the sporogonial or sporangial wall may thus be regarded as the most archaic part of the sterile system of the sporophyte. There is no direct evidence to show that the wall of *Riccia* has been the result of sterilization of sporogenous cells, but the analogy of the peridium of the Uredineae would suggest that view, which is strengthened by comparison with Algal forms. Further steps in advance among the Bryophytes exemplify two types of sterilization of

the centrally lying tissues: (*a*) that which, as the sporogonium elongates, involves the whole thickness of the sporogonium; (*b*) that by which single cells are diverted to a vegetative development.

The result of the first type is seen in most Liverworts and Mosses; as examples of its effect we see the sterile foot of the Marchantiaceae and Anthocerotcae, but it is more prominently shown in the Jungermannieae; in the latter there is no difference in origin or early stages between the central sterile region of the seta and the sporogenous mass of the capsule, and thus there seems good reason for holding that the former is the result of sterile development of a tissue which was in an earlier ancestry sporogenous. A similar view will apply with more or less clearness to other Bryophyta with stalked capsules.

Turning to the second type (*b*) of sterilization, in which single cells are diverted from the sporogenous to the sterile condition, though they are in origin the equivalents of those which remain fertile, we shall see that this is widely spread through the Archegoniatae, and upwards to the flowering plants. Among the Liverworts, *Oxymitra*, *Corsinia*, and *Boschia* show certain cells of the sporogenous mass which, though similar in origin, differ at an early stage from those which form spores: these commonly take the form of elaters, as in *Marchantia* and *Targionia*, &c., and are often distributed uniformly through the sporogenous mass.

A peculiar case is that of *Frullania*, in which the sterile cells appear as elongated trabeculae arranged with some regularity, and reminding one, as regards their position and attachment, of the trabeculae in the large sporangia of *Isoëtes*.

In other Liverworts these elaters are liable to be grouped together, and thus form continuous tissue-masses. This is seen to a certain extent in *Pellia*, where they form a columella-like body rising from the base of the capsule; but better in *Metzgeria* and *Aneura*, where the mass hangs down from the apex of the sporogonium. These would appear to be imperfect steps towards the formation of a columella in the centre of the capsule, by the grouping together of sterile cells,

which in other forms are separate. In the Anthoceroteae, however, a complete central columella is commonly found, as also in most of the true Mosses. In *Dendroceros* this is well seen, though it is stated by Leitgeb that it is absent in some small specimens of *Notothylas*. In writing on these plants, Leitgeb clearly recognized the probable origin of this columella by massing of sterile cells: he remarks, 'the sterile cells at first uniformly distributed through the spore-cavity, though connected together, first united at the axis to form a strand of cells.' I think there is good reason for accepting this conclusion of Leitgeb that sterile tissue-masses, lying internally, may have originated in certain cases by coalition of isolated sterile cells: the actual spore-producing tissue was thus relegated to a more superficial position, which it occupies in all the higher Archegoniatae. In such plants with a columella, the sporogenous tissue is referable in origin to a dome-like layer of cells—the archesporium: this is the case in the Anthoceroteae, in *Andreaea*, and *Sphagnum*. But in most Mosses the apex of the dome does not produce spores, the archesporium thus having the form of a cylinder open at both ends. If the ordinary Mosses were derived, as would seem probable, from types with dome-like archesporium, we should then see in the cylindrical archesporium the result of a partial sterilization involving the apex of the dome.

It is further to be noted that the whole of the archesporium of the Anthoceroteae is not devoted to spore-production; certain of the cells form sterile elaters in *Anthoceros*; in *Notothylas*, however, the sterile cells form a network which holds the spore-mother-cells in its meshes. From such a condition as this to that of complete septation would appear to be but a slight step.

But here we arrive at the Rubicon: for among the Bryophyta that apparently slight step is not taken, and it may even be a question whether complete septation ever was accomplished by plants really akin to our present Anthoceroteae. The distinction between the Bryophyta and Pteridophyta is most strongly defined by the facts that the sporophyte of the former has a concrete archesporium, and no appendicular organs,

while that of the Pteridophyta has discrete archesporia, and possesses appendicular organs. The origin of the Pteridophyta was certainly in the very remote past; it is therefore not surprising that traces of the line of their advance should be few and uncertain, and the hypotheses based upon them divergent. Before discussing these matters, however, I shall show that sterilization of potentially sporogenous cells, which is so easily recognized in the Bryophyta, is also common among Vascular Plants. In some cases the function of the arrested cells appears to be simply nutritive to the developing spores: in other cases they form permanent tissue-masses. In the sporangia of *Equisetum* only about 60 per cent. of the cells of the sporogenous tissue undergo the tetrad division: the rest—in addition to the tapetum—become disorganized, and their substance absorbed into the developing spores. In *Psilotum* and *Tmesipteris*, of the ill-defined sporogenous mass which is surrounded by no definite tapetum, only a small proportion of the cells produce spores, the rest being disorganized as in *Equisetum*. In *Ophioglossum* again, as has been recently shown by M. Rostowzew, and confirmed by myself, a broad peripheral band of the sporogenous tissue is disorganized, and in the tetrad stage numerous nuclei, which are ultimately absorbed, are seen in the protoplasmic matrix in which the tetrads float: these are derived partly from cells of the periphery of the sporogenous mass, partly from others distributed through it (see Rostowzew, Rech. sur l'Ophioglossum vulgatum, p. 28). These examples will serve to show that a partial sterilization is of common occurrence in the sporogenous masses of homosporous Pteridophyta.

Among the heterosporous plants it is also seen, but most obviously in connexion with the maturing of the mega spores, though it also occurs in the microsporangia. The case of *Isoëtes* is sufficiently well known: here bands of tissue, differentiated from the sporogenous mass, develop as the trabeculae: in this case their origin by sterilization of potential sporogenous tissue has been generally accepted. I have recently shown that somewhat similar rods of sterile tissue

are found in the large sporangia of *Lepidostrobus Brownii*, while in other types of *Lepidostrobus*, as already shown by Professor Williamson, the sterile masses take the form of longitudinal plates, which project far up into the cavity of the sporangium in the mature state. Unfortunately it is impossible to follow the development in these cases, but I can assert that in point of position and structure, these processes, when mature, are strikingly similar to the trabeculae of *Isoëtes*.

Turning for a moment to the megasporangia, the arrest of potential sporogenous cells is there a prominent and well-known fact. For instance, in *Selaginella* a single tetrad of megaspores is developed, and all the other sporogenous cells are arrested. A similar condition is found in other heterosporous Pteridophyta. Again, in Phanerogamic plants there is frequent evidence of sterilization of cells of a potential archesporium: among the Gymnosperms *Gnetum* is a well-known example, demonstrated first by Professor Strasburger. Again, among the Angiosperms a similar condition is found in the ovule of *Casuarina*: this case is particularly interesting since the potential embryo-sacs are not simply obliterated by the growth of the favoured one, but some develop into tracheides with thickened walls, a proof that permanent sterile tissue may be formed from potentially sporogenous cells. In certain Amentiferae also a similar condition has recently been demonstrated. Lastly, I would recall the case of *Rosa livida*, also described by Professor Strasburger.

It is thus seen that in megasporangia and ovules, sterilization of potential sporogenous cells is of frequent occurrence, the cells arrested being for the most part obliterated by the developing megaspores. In the pollen-sacs of Angiosperms a similar sterilization of single cells has been noted, and is, I doubt not, common enough. But in the anthers of certain Angiosperms there may also be seen a formation of complete septa of permanent sterile tissue, dividing the pollen-sacs transversely, in plants whose near allies have their pollen-sacs not septate. Such examples appear to me to show in the most conclusive way *that septa may be formed by a partial*

sterilization of the sporogenous tissue. In the Onagraceae the stamens of most genera of the order are of the ordinary quadrilocular type; but in the genera *Circaea*, *Gaura*, *Clarkia*, and *Eucharidium*, the four loculi are each divided transversely by one or more sterile septa: these septa may consist of only a single layer of cells having the character of tapetum, or of two layers, or even of four or more, of which the middle layers then resemble the tissue of the connective: an examination of the early states of development supports the conclusion that the septa result from sterilization of part of the sporogenous tissue, for in sections it is seen that the sporogenous cells, and those which will form the septa, originate from a common layer corresponding to the archesporium of normal anthers. A similar state of things has been described by Rosanoff, and by Engler in certain of the Mimoseae, in many of which it is well known that there are eight pollen-sacs (species of *Inga*, *Calliandra*, *Acacia*, and *Albizzia*), while in others, e.g. *Parkia*, the number may be much larger. These Engler (Pringsh., Jahrb. x. p. 289) recognizes as being all merely variations of the one fundamental type with one row of primary mother-cells of the pollen at each angle of the anther: certain cells of these rows, developing as sterile tissue, provide the septa by which the four typical pollen-sacs are partitioned into eight or more loculi. From these examples we might proceed also to those of *Viscum*, of some species of *Loranthus*, and of *Rhizophora*: as the result of developmental study of the latter it is stated by Warming (Engler, Jahrb. Bd. 4. p. 519) that the multilocular condition is easily explained by arrest of development of parts of the sporogenous tissue, and formation of sterile septa. Probably a similar developmental explanation will be found to apply in certain other cases, e.g. *Aegiceras*, *Phajus*, *Bletia*, and *Rafflesia*, all of which have multilocular anthers. Since such septation resulting from partial sterilization is thus shown to occur in pollen-sacs of Angiosperms, it will, I think, be futile to deny the possibility of its having occurred also in lower forms: the question therefore becomes one of *probability* only.

It will probably be remarked that this occurrence of

septation among Angiosperms is but sporadic: that it is merely an 'adaptive' character, and that the sporadic occurrence of it deprives it of systematic importance. In answer to such objections I would say that to me all permanent morphological characters are probably at one time or another 'adaptive,' though we may distinguish between those which are results of relatively recent adaptation and those which were relatively primitive. It may be that the same method of advance may have brought in its train important physiological advantages at one point of evolutionary history: but when repeated at a later period the advantages may have been less weighty: the result would be in the one case relative permanence, in the other less permanence. This I conceive to be the case for septation of sporangia in the homosporous Archegoniatae as compared with septation of anthers in Angiospermic plants.

But while we thus recognize that parts of the sporogenous tissue may form sterile septa, I have been able to show that the converse may also take place; viz. that tissue of a septum, which is normally sterile, may on occasions form spores. This occurs in certain abnormal synangia of *Tmesipteris*, and is, in my view, a reversion. In Fig. 161 of my memoir in the Philosophical Transactions[1], a synangium of almost normal form is seen: but though the position of the usual septum is clearly indicated, the cells there are already assuming the character of tapetum or of sporogenous cells, as is more clearly seen in Fig. 162, where they are represented in a drawing on a larger scale. This conversion of the sterile septum into sporogenous tissue is more conclusively shown in Fig. 164, in which the development is slightly more advanced, while Fig. 165 represents a part of the contents about the line (x) where the septum should normally be: here it is seen that sporogenous cells form a continuous chain across the line, thus conclusively proving that the normally sterile septum has become sporogenous.

The conclusion to be drawn from such observations, together

[1] See Proc. Roy. Soc., No. 326, 1893.

with the facts of formation of septa above noted, will be, that *there is no fundamental difference between sterile tissue of a septum and sporogenous tissue, since both appear to be mutually convertible.*

Lastly, I would point out that the arrest or sterilization may involve a whole sporangium. If the strobili of species of *Lycopodium* or *Selaginella* be examined, it will be found that, passing downwards from the base of the strobilus, sporangia are to be seen in the normal position, but successively of smaller size, till a small sterile group of cells is all that represents the sporangium: finally the sporangium disappears altogether. A similar state of things is found at the apex. Again, in such species as *L. Selago* there are successive sterile and fertile zones, which graduate off into one another through zones showing such successive series of abortive sporangia as those above noted. Similar imperfect bodies (synangia) are frequently found at the limits of the fertile zones in the Psilotaceae, while the fertile spike of *Ophioglossum* may also be represented by a small non-fertile body. How are these facts to be regarded from an evolutionary point of view? Are the small non-fertile bodies nascent germs of sporangia, or are they vestigial? As a third alternative it may be suggested that they have no phylogenetic bearing whatever; but considering the frequency of their occurrence, and the gradual steps of their arrest, I do not think this last view to be a probable one. I think we can only consider them to be vestigial organs: potential sporangia, arrested, and in the down-grade of development. Their occurrence is doubtless very closely connected with the physiological position of the plant which bears them, but the recognition of this does not in any way explain away the interest which attaches to their frequent presence. The leaves which subtend the sporangia (sporophylls) differ in many species of *Lycopodium* from those which do not (vegetative leaves), though their relation to the axis is the same: in passing from the strobilus to the vegetative region a gradual transition from sporophylls to the vegetative leaves is seen, and it proceeds parallel with the arrest and final disappear-

ance of the sporangia. If the arrested sporangia be accepted as vestigial, then the correlatively larger foliage-leaves which subtend them must be regarded as sterilized sporophylls, and the conclusion follows that *in some cases at least foliage-leaves are sterilized sporophylls.*

Before leaving this subject it may be remarked that a similar view has been applied by Prantl and others to the stamens and perianth-whorls of Ranunculaceae, &c., viz. that the latter owe their origin to sterilization of staminal members. It seems not unlikely that this view may be applicable also in many other cases among the higher plants.

We have thus arrived at the position that sterilization of potential sporogenous tissue is a common phenomenon, recurring frequently throughout the Archegoniatae and Phanerogams. We find it affecting single cells, groups of cells, or even whole sporangia. In certain cases the sterile cells may remain isolated, or be absorbed by the developing spores, thus appearing to serve a directly nutritive function: in other cases permanent sterile rods (trabeculae, or columellae) or plates of tissue may be formed, and there is reason to think that a grouping together of sterile cells may have resulted in certain cases in the formation of sterile tissue-masses (Anthoceroteae). In others, again, complete septa may partition off a previously concrete potential sporogenous mass into isolated portions: such tissue-masses as trabeculae and septa may serve the purposes of mechanical support, and of assistance in bringing nutrition to the masses of developing spores. We have further learned from the resumption of spore-production by certain sterile septa, coupled with the facts of sterilization, that there is no fundamental difference between sterile septa and fertile tissue, either being convertible into the other. With these conclusions before us, drawn from both higher and lower forms, we may now address ourselves to the problem of forming some idea of the probable methods of morphological advance of the simpler homosporous Vascular Cryptogams. On grounds of comparison it has been generally held that they originated from some Algal-Bryophytic

ancestry, and I see no sufficient reason for doubting this view. I am fully impressed by the great remoteness of their origin in point of time, and by the absence of a sufficient geological record which would throw direct light on their descent. There appear, however, to be two other foundations for opinion on this question, viz. close examination of the individual development and physiological position of the Vascular Cryptogams, and comparison of the Bryophyta. We are, I think, bound to use our knowledge of the Bryophyta as a guide to an idea of how the more complex Vascular Cryptogams came to be. It may be a question how far the Bryophytes, as we see them now, illustrate what was the actual line of descent of Vascular plants: the similarities may be merely those of analogous forms: but we have among other living plants no better guide, and it would be nothing short of culpable neglect to leave aside the line of analogy which the study of the Bryophytes suggests. And as we see in them a sequence of forms starting from those in which the sporogonium is of simple form and almost entirely (in certain Algae entirely) devoted to the duty of spore-production, so we may believe that the more complex Vascular Cryptogams had a similar origin: this is in fact substantially the current opinion. We may accordingly contemplate the origin of plants with discrete archesporia and appendicular organs, from plants with concrete archesporium, and simple form of the sporophyte. First, we may consider the biological advantages which must have followed from the advance of complexity. We have already recognized the advantage gained in archegoniate plants by increased output of spores, and noted the advance among the Bryophyta, and some of the simple devices adopted by them to meet the demands of nutrition and dispersal of their numerous spores. Throughout that series, however, the nourishment of the sporophyte is mostly received at second hand, the application of its sterile tissues to the vegetative office of nutrition being only imperfectly carried out. In the Pteridophyta, however, the nutrition is supplied by the sporophyte itself, after the first embryonic

stages are over. The advantages of direct nutrition, and of perennation of the vegetative system are, from the point of view of spore-production, obvious enough, and a greatly increased output of spores became thereby possible.

The second point, viz. the substitution of discrete archesporia for one concrete one would also be a great biological advantage: as the size of the single sporogenous mass increases, the difficulty of supply of food to all the developing spores makes itself increasingly felt, while further the risk of mechanical damage becomes more serious: for during the semi-fluid condition of the contents of the sporangium, where all the sporogenous cells are separate from one another, a large sporangium is mechanically ill-protected, while a single puncture by animal or other agency would ruin the whole. Again, separate loculi may be matured in succession, thus not making a simultaneous demand for nutrition, while keeping up a supply of spores for an extended period. From the point of view of nutrition and protection, therefore, septation would appear to be a biological advantage.

Thirdly, from the point of view of dispersal, a projection of the sporangia beyond the general surface is clearly a gain: the mechanical difficulties of scattering the spores from projecting sporangia being much less than from deeply seated ones. Thus on various grounds we should be prepared to expect septation and separation of sporangia to appear in increasing degree in an ascending evolutionary series.

We shall next inquire if among living Vascular Cryptogams there is any evidence which would support the idea that septation has taken place: and the answer is to be found in the frequent occurrence among them of synangia, such as those of *Tmesipteris*, *Psilotum*, *Danaea*, *Marattia*, *Kaulfussia*, *Ophioglossum*. Moreover, the sporangia, when distinct from one another, are so grouped in many Pteridophyta as to suggest a common origin from a synangial body by separation and rounding off of the individual sporangia: this is the case with the sori of many Leptosporangiate Ferns. The development of the sporangiophore of *Equisetum* suggests a similar

view as the explanation of its origin also. Hitherto the converse view has commonly been entertained, viz. that synangia are the result of coalescence of sporangia in the course of descent from an ancestry with separate sporangia; in fact, the whole morphology of homosporous Pteridophytes has been dominated by the belief that the Leptosporangiate Ferns are the nearest of vascular plants to the Bryophyta. I have already stated at length elsewhere my reasons for thinking that view to be ill-founded (Annals of Botany, Vol. V. p. 109): however firmly convinced any readers may be of the correctness of the belief that the Leptosporangiates were the most primitive Ferns, and the Ferns the most primitive of vascular plants, I would ask them for the moment to relinquish that opinion, and contemplate with me an alternative view.

First, I would state the opinion that, in the course of evolution, simple and small-leaved forms preceded complex and large-leaved forms; accordingly, unless there be strong reasons against it, we shall be prepared to seek among small-leaved, strobiloid, homosporous Pteridophyta for those which reflect most nearly the primitive condition.

Secondly, it would appear that, unless there be strong evidence to the contrary, synangia should be recognized as the result of septation. The examples above cited from the anthers of Angiosperms were treated by writers on the subject in the right way; partly from comparison of allied forms, and partly from the study of development, it was concluded that the anthers had become septate owing to a partial sterilization of potential sporogenous cells. The same has been concluded in the case of the trabeculae of *Isoëtes*. A similar course should be taken with the synangia of Psilotaceae, Filicineae, and Ophioglossaceae; but I would premise that in plants where, as in these, the meristems are not disposed in definite strata, the recognition of a potential archesporium as a definitely limited and continuous band of tissue must not be too rigorously demanded before the synangia be admitted as results of septation. The facts which have been acquired relating to the Psilotaceae have already been stated at length

elsewhere (Phil. Trans. 1894), and those relating to the Ophioglossaceae are nearly mature; in both cases the developmental facts support a view of septation. It appears to me that morphologists have in the past been too ready to take refuge in hypotheses of reduction, as applied to the homosporous Archegoniatae. It has been assumed, with, as I think, too little reference to the biological position of the organisms, that synangia were the result of coalescence of originally distinct sporangia. I hold that, on considerations of development, comparison, and biological position, there is good reason to believe that certain at least of the plants bearing synangia were on the up-grade of evolution, and *that septation has been a factor in producing them as we now see them.*

There is one further factor which it will be necessary to discuss, as contributing to the advance of the sporophyte from similar beginnings, viz. the origin of appendicular parts. The origin of the root may be dismissed as not directly affecting our present discussion, and there will remain the question of origin of such parts as are included under the terms sporangiophore, sporophyll, and foliage-leaf. It is conceivable that there may have been various modes of origin of such parts, and that they are not all truly comparable as regards their descent; but I think there is good reason to believe that at least one mode of origin was *by a process of eruption from a hitherto smooth surface.* This suggestion is in no way subversive, but falls in with observed facts of the origin and development of leaves in vascular plants generally; whether we take the strobilus or the vegetative shoot, the ontogenetic origin of the appendicular parts might be described as eruptive. What I suggest is, that the phylogenetic history of sporophylls was similar to the history of the individual as we now see it. It is easy to bring forward examples of analogous eruption of new parts of a higher order from the smooth surfaces of other parts; for instance, in the development of simple foliage-leaves the margins are occupied by smooth wings; in compound leaves, however, these smooth surfaces show an eruptive

upgrowth of papillae, which develop into the pinnae; moreover, the whole of the wing-surfaces is not occupied by the eruptive growths, so that the smooth and the eruptive conditions may often be seen at different regions of the wings of the same foliage-leaf. Again, in certain flowers, as in the Hypericaceae and Myrtaceae, the originally smooth surface of the staminal growths shows an eruptive development of numerous stamens. Thus, whether in the vegetative or floral regions, eruptive formation of new parts from a smooth surface is known. I have elsewhere contended for a consistent morphological treatment of the shoot throughout (Phil. Trans. 1884, Part II). Botanists recognize the appearance of pinnae by an eruptive process on the margins of the leaf, and would, I presume, admit that simple leaves preceded compound ones. What I suggest now is that the origin of the sporangiophore or sporophyll in the descent of Vascular Cryptogams from some simpler Archegoniate forms was by a similar eruptive growth from the smooth surface of a body of the nature of a sporogonial head.

Having thus cleared the ground, and having recognized as possible factors in the advance a process of septation by formation of sterile septa from a previously continuous archesporium, and an eruption of the surface so as to produce appendicular organs, I will now briefly state certain views at which I have arrived as regards the morphology of the Pteridophyta, but the detailed production of facts will have to be given elsewhere.

Fixing attention mainly on the spore-producing region, since for reasons already noted the sporogenous tissues are to be regarded as primary, we see on the one hand among the Bryophyta that the spores are produced on sporogonial heads, with a central sterile columella, surrounded by a continuous archesporial layer, and protected by an external wall. Selecting some small-leaved, strobiloid Vascular Cryptogam, we see the strobilus performing the same function of spore-production, but with different superficial conformation. Centrally is the sterile tissue of the axis, the internal part of which may be

compared with the columella; the continuous archesporium of the Bryophyte is replaced by the discrete archesporia of the Pteridophyte, while these are carried out on appendicular organs, the sporangiophores. Thus the strobilus would appear to be the correlative of some body like a sporogonial head, in which the archesporium is septate and borne outwards on eruptive growths. Applying these ideas to the strobilus of *Equisetum*, we see the probability of them reflected in the development. The surface of it when young is almost smooth: the isolated cells which are to form the archesporia, can be recognized almost as soon as the surface becomes undulated by the eruption of the sporangiophores: these cells are proportionately nearer the surface than in the Bryophyta: but it has already been pointed out that the very formation of the columella was a step in the direction of relegating the spores to a superficial position, while such a position is necessary for the dispersal of the spores from small loculi. I do not mean to suggest that by such comparisons as these the hypothesis of origin of a strobilus from a body of the nature of a sporogonial head can be proved: the comparison may be, and indeed probably is, merely a tracing of analogies in parts which have advanced along somewhat similar lines: and our endeavour is, as explained at the outset, not so much to trace homologies as to recognize the methods of advance in archegoniate plants: the chief points which have been recognized thus far, and are believed to have been the important factors in advance are (1) *sterilization of potential sporogenous tissue*, (2) *formation of septa*, (3) *relegation of the spore-producing cells to a superficial position, and* (4) *eruption of outgrowths (sporangiophores) on which the sporangia are supported.*

In the Lycopods also the whole strobilus may be recognized as the result of similar methods of advance: regarding *Phylloglossum* as probably their simplest representative, we see that the plant consists of the protocorm, bearing protophylls, which may have been the result of direct vegetative outgrowth from the protocorm. Borne upon an elongated

peduncle is the simple strobilus: its conformation does not differ very materially from that of *Equisetum*, beyond the smaller number of the sporophylls, and the fact that each bears but one sporangium upon its upper surface, instead of a number affixed all round. From *Phylloglossum* the advance to the more complex Lycopods can best be understood by comparing them in the young state: the similarity of the young plant of *L. cernuum* to that of *Phylloglossum* is so striking that it has been recognized by various writers: the chief difference lies in the leafy bud which in *Lycopodium* replaces the strobilus of *Phylloglossum*, and grows on into the *Lycopodium* plant as we know it. On our hypothesis we may understand how continued apical growth of the strobilus would lead to its elongation, and increase in number of sporophylls, sporangia and spores: branching of the strobilus has been seen in *Phylloglossum*, but is a more prominent factor in *Lycopodium*. Meanwhile, in order doubtless to increase the vegetative system necessary for the nutrition of the larger number of spores, the lower sporangia would be arrested, the subtending sporophylls developing as foliage-leaves. In species such as *L. Selago* alternating sterile and fertile zones appeared, in others as *L. clavatum* the whole lower part of the plant is vegetative, while the strobili occupy the ends of special branches. It is thus possible to recognize the shoot-system of living species of *Lycopodium*—exclusive of the protocorm and protophylls—as the result of continued growth and branching of a strobilus, the lower parts of which have become sterile.

Within the genus *Lycopodium*, but more obviously in *Lepidostrobus* and *Isoëtes*, differences of size of the individual sporophylls, and of bulk of the individual sporangia, are seen: the latter show very large sporangia as compared with *Lycopodium*, and we may recognize as one of the methods of increase of the output of spores, the increase in size of the individual sporangium. But with this would come again the difficulty of nutrition, and danger of damage. These are partially met in *Isoëtes* and *Lepidostrobus* by processes of

sterile tissue projecting upwards into the sporangium, though more efficiently in the Psilotaceae by formation of complete septa. But the septation which is there seen in a minor degree becomes a more prominent feature in the Ophioglossaceae, a family which on general grounds of comparison of both generations I believe to be allied to the Lycopods. I cannot here enter into details of the evidence (see Phil. Trans. 1894), suffice it to say that I still consider the facts sufficient to support the conclusion advanced some years ago: that septation has resulted in the production of the fertile spike of *Ophioglossum* from a sporangium of Lycopodinous type. The other genera *Botrychium*, and *Helminthostachys* may be regarded as showing further steps in the separation of distinct sporangia, the latter exhibiting also an eruption of sporangiophores, laterally on the fertile spike: a repetition in fact of the same process as we have recognized in the origin of the strobilus itself. This great elaboration of the body which appears to correspond to the Lycopodinous sporangium, marches parallel with the increase of the subtending sporophyll: in this series, which I believe to be an advancing series, I think we may thus see how characteristically large-leaved forms, with relatively few leaves expanded in slow succession, may have arisen from strobiloid forms with numerous small leaves.

This leads to the third large series of Pteridophyta, viz. the Ferns: I think it not improbable that they, with their large leaves and numerous sporangia, originated from some smaller-leaved strobiloid ancestry. On grounds already explained elsewhere (Annals of Botany, Vol. V, p. 109) I have concluded that the Eusporangiate Ferns were probably the more primitive, and of these perhaps *Danaea* the most so. It is not difficult to understand how, on a hypothesis of septation of sporangia spread out over the surface of an enlarging sporophyll, the peculiar sorus of that Fern might originate: from such a type, by divers methods of isolation of the sori, and separation of the sporangia, the various forms of Leptosporangiate Ferns may have been derived. So far then from accepting the latter as the primitive types of

vascular plants, I should rather regard them as later derivative forms, characterized by an extravagant growth of the leaf and its appendages.

The chief points which the Ferns have in common with the Ophioglossaceae are the large, often compound leaf, and numerous, often separate, sporangia. It is easy to imagine the origin of these two families by parallel development from a smaller-leaved ancestry: in the one case the extension and septation of a definite sporangium of Lycopodinous type would result in the so-called fertile frond of the Ophioglossaceae, this enlarging *separately from the subtending leaf*: in the other the spore-producing body *would throughout be closely connected with the enlarging leaf-surface* and be spread out over it, as we see to be the case in *Danaea*. The method of advance in complexity would be virtually the same in both cases, viz. by septation, and subsequent separation of the several sporangia. But evidence on such points as these is but of the slenderest.

If this theory be applicable to the strobilus of Vascular Cryptogams, it should also be so to the flower of Phanerogams. At present I do not propose to pursue the matter further in this direction, beyond saying that I see no valid objection in the way, while the recognition of the Phanerogamic flower as a strobilus of ultimate origin like that of Vascular Cryptogams would make certain difficulties of floral morphology appear less serious. But though the Phanerogamic flower be accepted as the homologue of the strobilus, it must never be forgotten that while the homosporous strobili are entirely non-sexual, the flower of Angiosperms is in its development intimately connected with the sexual function. The presence of this important factor in the one, and its absence in the other makes it difficult to draw physiological comparisons between them as regards the conditions which would produce or modify them.

In previous attempts to explain the origin of the complex strobilus of vascular plants, some idea of terminal branching similar to that branching which is occasionally seen in abnormal

Moss-sporogonia has been held. The result of this would be that the apex of the sporophyll would compare with the apex of a sporogonium, or of some branch of it. In suggesting that the whole strobilus is the equivalent of some body of the nature of a sporogonial head, clearly the apex of the one will correspond to the apex of the other, while the eruptive members will be mostly or all lateral.

In entertaining this theory, we shall necessarily part company with the old views of metamorphosis, and I must state, with all distinctness, that the opinion that the strobilus is a result of modification of a vegetative shoot is, to my mind, incompatible with our present views as to descent of plants constantly maintaining an antithetic alternation, in which spore-production was a regularly recurring event. On grounds of comparison the converse would appear to be more probable; but in any case there seems no sufficient reason to think that the strobilus, or indeed the Phanerogamic flower, ever was a foliage-shoot.

In conclusion, I would not be understood to take up a dogmatic attitude as regards any of these questions. While recognizing septation as one mode of origin of separate sporangia, I would not deny that other modes may have occurred. Similarly, while tracing the origin of some vegetative leaves to the sterilization of sporophylls, I do not deny other sources of origin of foliage-leaves. Nor do I put forward this theory of the strobilus except in the most tentative way. The stability of a theory is to be measured by the extent of the facts which it will satisfactorily cover. I think those who carefully consider the matter will find that a very large number of facts will be susceptible of more ready interpretation in accordance with it. I submit the suggestion as a working hypothesis which has been before my mind during some years of active investigation.

The main points of the theory may be briefly stated as follows:—

1. Spore-production was the first office of the sporophyte, and the spore-phase has constantly recurred throughout the descent of the Archegoniatae; the spore-bearing tissues are to

be regarded as primary, the vegetative tissues as secondary, in point of evolutionary history.

2. Other things being equal, increase in number of carpospores is an advantage; a climax of numerical spore-production was attained in the homosporous Vascular Cryptogams.

3. Sterilization of potential sporogenous tissue has been a wide-spread phenomenon, appearing as a natural consequence of increased spore-production.

4. Isolated sterile cells, or layers of cells (tapetum) served in many cases the direct function of nourishing the developing spores, being themselves absorbed during the process.

5. By formation of a central sterile mass (columella, &c.) the spore-production was, in more complex forms, relegated to a more superficial position.

6. In vascular plants, parts of the sterile tissue formed septa, partitioning off the remaining sporogenous tissue into separate loculi.

7. Septation to form synangia, and subsequent separation of the sporangia, are phenomena illustrated in the upward development of vascular plants.

8. Such septation may have taken place repeatedly in the same line of descent.

9. The strobilus as a whole is the correlative of a body of the nature of a sporogonial head, and the apex of the one corresponds to the apex of the other.

10. Progression from the simpler to the more complex type depended upon (*a*) septation, and (*b*) eruption to form superficial appendicular organs (sporangiophores, sporophylls) upon which the sporangia are supported.

11. By continued apical growth of the strobilus, the number of sporophylls may be indefinitely increased.

12. The sporophylls are susceptible of great increase in size, and complexity of form; in point of evolutionary history, small and simple sporophylls preceded large and complex ones.

13. In certain cases foliage-leaves were produced by sterilization of sporophylls.

NOTES.

THE SIEVE-TUBES OF CALYCANTHUS OCCIDENTALIS (Hook and Arn.)—In *Calycanthus*, De Bary describes the four cortical bundles with reversed orientation, and with regard to their phloëm he remarks, 'It only consists of soft bast, and, in the main at least, of parenchymatous elements; sieve-tubes remain still to be sought for[1].' Herail, who subsequently studied the origin and development of these bundles, states[2] that instead of being cortical they are derived from the pericycle. The hypocotyledonary stem is described as possessing four masses of phloëm, but no cortical bundles. The latter become separated from the phloëm of the bundle-ring about the middle of the first internode. Herail states that sieve-tubes are present in this part of the stem, but he makes no mention of them in connexion with the cortical bundles.

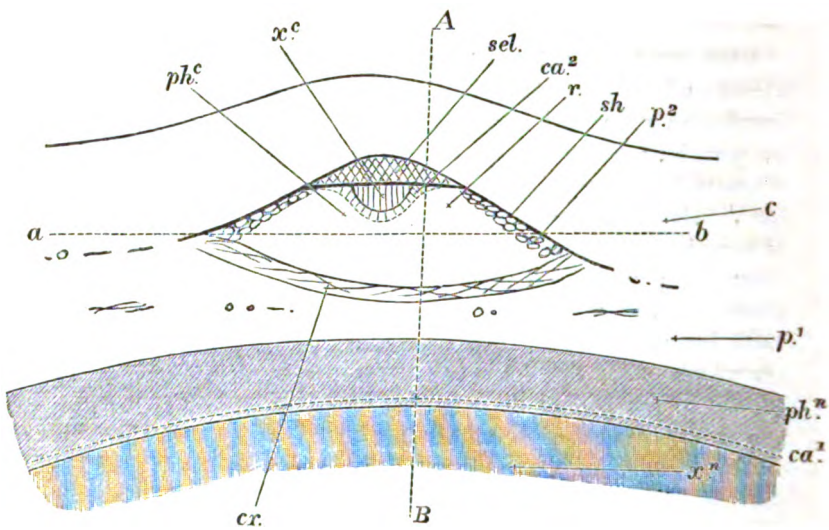
Young stems of *C. occidentalis* were examined and the greater part of the phloëm in the cortical bundles was seen to consist of sieve-tubes. On comparing the bast of these bundles with that of the bundle-ring it was found, as a matter of fact, that, for equal areas, the sieve-tubes in the former were far more numerous than in the latter.

The following details may be added. A transverse section shows the cortical (pericyclic, according to Herail) bundle embedded in the cortex, and separated from the normal phloëm by a very variable number of parenchymatous cells with large interspaces, see woodcut p^1. Its shape is that of a sector of a circle with the angle towards the periphery, and the arc near the bundle-ring, while the radii diverge at an angle of about 120°. A semilunar band of sclerenchymatous

[1] De Bary, Comp. Anat. Eng. Ed. p. 584.

[2] Herail, 'Recherches sur l'anat. Comp. de la Tige des Dicotyledones,' Ann. Sci. Nat. Bot. 7 Sér. Tome 2, p. 236.

fibres (*scl.*) marks the position of the angle, and the radii are formed by single rows of sclerotic cells (*sh.*), of the same shape and size as those of the cortical parenchyma; in each of these cells the wall nearest the bundle is much thickened, strongly lignified, and deeply pitted. The arc of the sector is denoted by a prominent band of crushed phloëm (*cr.*), often four or five cells thick, in which the sieve-plates can be distinguished. The xylem forms a small segment of a circle



WOODCUT 2.

Diagram of cortical bundle of *Calycanthus occidentalis*, Trans. Sect. of young stem. x^n Xylem of bundle-ring. ph^n. Phloëm of ditto. p^1. Parenchyma between the cortical bundle and the normal phloëm. *cr.* Crushed phloëm of cortical bundle. ca^1. Cambium of bundle-ring. *C.* Cortex. p^2. Parenchyma of cortical bundle. *scl.* Band of sclerenchyma. *sh.* Sclerotic sheath. x^c. Xylem of cortical bundle. ph^c. Phloëm of ditto. ca^2. Cambium of ditto. *AB* and *ab* indicate the position of the radial and tangential sections referred to. *r.* Recesses between xylem and sclerotic sheath.

with its base applied to the middle of the base of the band of sclerenchyma (x^c). The length of the base of the former is one-third to one-half that of the latter. A cambial zone (*ca.*) borders the convex edge of the xylem, while the whole remaining space is occupied by phloëm (ph^c).

Between the inner (phloëm) edge of the cortical bundle and the

normal phloëm there is a band of large parenchymatous cells with dense contents and large interspaces. Sometimes strands of sclerotic cells and fibres occur here.

The phloëm of the bundle-ring consists chiefly of parenchymatous cells densely packed with starch and masses of brown crystalline substance, the latter being aggregated chiefly in, or near, the cambial region.

Large rectangular secretory sacs are distributed irregularly through the cortex and bast region of the bundle-ring, but are absent from the cortical bundles. The tissues of the normal phloëm are much more loosely packed than those of the cortical bundle: while there are few interspaces in the latter, they are numerous and large in the former and are even found close to the cambium.

Tangential sections through the phloëm-portion of the cortical bundle just centrally to the reversed xylem (along the line *ab*) show on either side:—

(1) The one-sided sclerotic cells above mentioned (*sh*).

(2) Within these, also on both sides, are one of four rows of parenchymatous cells (p^2) not unlike those of the cortex, and with dense starchy contents.

(3) The whole of the remaining space is occupied by large sieve-tubes and their companion-cells together with a few cambiform elements. As a rule, from sixteen to thirty sieve-tubes may be counted here. They are also found in the recesses (*r*) on either side of the xylem. The companion-cells are short, but very clear, and with evident nuclei. The sieve-plates are very prominent even in unstained sections; they are mostly transverse, but some are oblique and others nearly vertical. All of them are simple, and no cross-connexions were seen here. When a corresponding tangential section passing through the phloëm-ring of the central cylinder is compared with the above, it is seen that the sieve-tubes are very few in number: sometimes parenchyma only can be seen. In other parts from one to four sieve-tubes occur close together. Their course is strikingly irregular, and in many places it can be seen that the segments belong to originally separate rows of cells, union being effected by a mutual curving of the cells to one another.

Unlike the phloëm of the cortical bundles there are numerous cross-connexions very often quite short, but even then showing companion-cells. The sieve-plates are inclined in all directions, very few being

transverse, while occasionally a sieve-plate may be seen in a lateral wall near the end of a segment. The segments are very variable in length, some being equal to about six cambiform cells while others do not exceed the length of one of these.

A radial section through the peripheral sclerenchymatous band, already referred to (along line *AB*), just where it passes out into the sclerotic sheath, shows about two rows of cambial cells, then from seven to nine sieve-tubes with their companion-cells. These abut directly on the crushed protophloëm-band (*cr*) of the cortical bundle. When a radial section is made through the wood, as the latter projects into the phloëm-mass, there seems to be a larger number of cambiform elements here, and the number of sieve-tubes is often reduced to four or five.

In the part of the section between the bundle and the phloëm can be seen first, a number of large parenchymatous cells, then sometimes a band of sclerotic cells, or of fibres, and occasionally narrow bands of crushed elements. These bands also pass in a very interrupted line round the bundle-ring between the cortical bundles.

In the phloëm itself can be seen a mass of parenchyma with one to four sieve-tubes. Their course here is far more regular than it appears to be in tangential section.

Sections were cut at points between the cortical bundles, but it was not found that sieve-tubes in the normal phloëm were more numerous here than opposite the cortical bundles.

It thus appears that the greater part of the sieve-tube system of the stem is located in the cortical bundles. This fact makes the structure and development of these bundles still more interesting.

J. LLOYD WILLIAMS.

ROYAL COLLEGE OF SCIENCE, LONDON.

THE INFLUENCE OF LIGHT ON DIASTASE[1].—It was shown by Brown and Morris in their researches on the physiology of foliage-leaves that the amount of diastase that can be extracted from foliage-leaves varies considerably in the course of twenty-four hours, being greatest after a period of darkness and relatively less after long illumination.

Marshall Ward has shown again that the solar rays exercise a very

[1] Abstract of a paper read before the British Association at Oxford, August 1894.

destructive influence on certain enzymes that are excreted by various bacteria.

From a consideration of these two facts it seems possible that light may be destructive to the ordinary enzymes of the differentiated vegetable organisms. Some experiments have been made by the writer on this point, and though these are very far from complete the results obtained so far seem to have sufficient interest for them to be communicated to the British Association.

Diastase being an enzyme which is easy of extraction and capable of easy quantitative estimation, has been the one selected. It has been prepared for the experiments from ordinary malt. A series of experiments has also been carried out on saliva.

The mode of experiment has been to prepare some extract of malt by infusing the ground grains with water or salt solution, and to expose half of the quantity to strong light, either solar or electric, for varying times. Then measured quantities of the exposed and of the unexposed halves have been allowed to act upon thin starch-paste (1%) at the temperature of digestion, at about 40° C. or at the laboratory temperature. During the action its progress has been tested from time to time by adding a drop of each digestion to a drop of iodine and noting the resulting colour. When digestion has been well advanced, both tubes have been boiled with excess of Fehling's fluid and the resulting precipitate collected, washed, combusted in a platinum crucible and weighed as Cu O.

The extracts have been kept free from bacteria by using ·2 per cent. KCy as an antiseptic.

The preliminary experiments were made with an ordinary fairly strong extract of malt. Details of three are subjoined.

Experiment 1. Fairly strong malt extract, half exposed in white glass test-tube to sunlight during two days. The other half exposed to same rays, but covered with opaque screen.

Afterwards both tested with starch-paste. When digestion had proceeded for seventeen minutes at temperature 20° C. it was completed in the control-tube, but was incomplete in the one containing the extract that had been exposed to light.

Experiment 2. Weaker extract, exposed to diffused light during five days, receiving during that time about twelve to fifteen hours sunshine.

Subsequent conditions as in experiment 1. The digestion was

completed in thirty-five minutes in the control-tube, but was then incomplete in the other.

Experiment 3. Similar extract. Both exposed to diffused light for eleven days; one tube covered with opaque screen: the two tubes side by side in a beaker of water to ensure uniformity of temperature. After this exposure both allowed to act separately on starch as before. Titrated with Fehling's fluid after forty-five minutes digestion at 40° C.

The digestion with the extract kept in the dark gave a reduction of ·23 gm.; that with extract exposed to light gave only reduction of ·092 gm. Cu O. The quantity of extract alone used in both cases reduced ·083 gm. Cu O. Deducting this from each, D reduced ·147, L only ·009 gm. Cu O; showing a great impairment of the diastase.

The next set of experiments was made using the light from a strong electric arc-lamp. The solutions of the enzyme were prepared by precipitating the diastase from the extract of malt by means of 30 per cent. alcohol. The precipitate was rapidly collected by filtering under pressure, and was dissolved in ·2 per cent solution of K Cy. When rapidly done, this process yielded a nearly colourless solution, which had great diastatic power and which was free from sugar and contained a mere trace of proteid matter.

The first experiments were made by suspending a glass cell in which the extract was contained at a distance of two feet from the arc-lamp, keeping a control quantity in the dark.

Contrary to expectation, the diastase was found to be increased in amount by the exposure; in one case from twenty-nine to thirty-two; in another from three to four. Spectroscopic examination of the glass showed that it cut off a large proportion of the violet end of the spectrum.

Experiments were then made, avoiding the use of glass, employing either agar films, in which the enzyme was suspended, or quartz cells containing the fluid extracts.

The light was found under these conditions to retard the action, as in the case of the solar rays of the first set of experiments.

With the agar films the result was D : L : : 4 : 1.

With the quartz cell it was D : L : : 12 : 5.

The beam of light was thus found to have two effects. The rays of the violet end of the spectrum were markedly prejudicial, those of the red end were on the whole beneficial.

Similar results were yielded with saliva.

To confirm this, an experiment was made exposing some extract to the light in a quartz cell, and simultaneously some of the same in a glass cell, keeping a third quantity in darkness. The results were:— D gave a reduction of ·052, G one of ·055, and Q one of ·037 gm. Cu O.

Q was retarded in the proportion of 52 : 37; G was accelerated in that of 55 : 52.

The colouring matter of the barley-grain was by further experiments shown to have a certain power of protecting the diastase from the deleterious action of the violet rays, whether it was dissolved in the extracts used, or whether it was used separately as a screen placed before the cells in which the exposure to the electric arc was made. The screen in the latter case was contained in a quartz cell superposed upon the quartz cell containing the extract.

The results of the experiments so far point to the following conclusions:—

1. Light, whether solar or electric, exercises a destructive effect upon diastase.

2. The deleterious influence is confined to the rays of the violet end of the spectrum, the others being slightly favourable instead of destructive.

3. The colouring matter of the barley-husk acts as a screen preserving the diastase from the destructive effect of light.

The destructive influence continues after the exposure to light is discontinued, the exposed solution getting weaker and weaker till it had no diastatic property. The part of the solution kept in darkness maintained its diastatic power unimpaired for more than a month, by which time the exposed part, kept in darkness after its period of exposure, possessed no power to act upon starch.

J. REYNOLDS GREEN, London.

NUCLEOLI AND CENTROSOMES.—It may be of interest to state briefly here the results of some studies on these structures carried on during the past winter in Prof. Strasburger's laboratory at Bonn. A somewhat fuller account, with a plate, has been published elsewhere[1].

Although the occurrence of nucleolar substance in the cytoplasm has been observed by earlier writers, little significance has been

[1] Berichte der Deutschen bot. Gesellsch., Bd. XII, Heft 5, pp. 108–117.

attached to the phenomenon until very recently. Within a year, two investigators have attempted to base somewhat important views upon the observation of nucleolar masses beyond the limits of the nucleus during karyokinesis. Zimmermann[1] attempts to demonstrate that the occurrence of nucleoli in the cytoplasm during nuclear division is a typical phenomenon, and, regarding the nucleoli as permanent and definite organs, he holds that they are thrown out during division and again taken up into the daughter-nuclei resulting from the division. Thus he comes to regard all nucleoli as derived from previous ones and, extending the generalization accepted for the cell and the nucleus, to write 'Omnis nucleolus e nucleolo.'

Karsten[2] appears to have observed a similar condition in spore-mother-cells of *Psilotum*, and to have been led by the manner of their occurrence to regard the nucleolar masses as identical with the centrospheres, first described for plant-cells by Guignard; though he admits that they occur far less definitely and regularly than Guignard has described.

It is well known that the bodies known as nucleoli may be readily distinguished from other constituents of the vegetable cell by means of suitable staining media, so definitely as to leave little room for uncertainty. They take a blood-red colour on exposure to a mixture of fuchsin and iodine-green, and subsequent treatment with alcohol containing iodine and acetic acid[3] intensifies the selective staining. For fixing material for cell-studies nothing is better, as a rule, than alcohol of 95 per cent. or higher.

The studies of various tissues of several plants has led me to the conclusion that the extrusion of nucleolar substance during karyokinesis, far from being a normal occurrence, is a very exceptional one; and that the persistence of recognizable masses of this substance during division is so unusual as either to have a pathological significance or to indicate incomplete or unsatisfactory fixation of the material. The nucleoli usually disappear before the breaking down of the nuclear membrane and reappear after the constitution of the daughter-nuclei. Their entire behaviour is such as to justify but one

[1] Ueber das Verhalten der Nucleolen während der Kerntheilung: Beitr. z. Morph. u. Phys. d. Pflanzenzelle, Bd. II, Heft 1 (1893).

[2] Ueber Beziehungen der Nucleolen zu den Centrosomen, &c., Ber. d. D. bot. Ges., Bd. XI, p. 555 (1894).

[3] Zimmermann, loc. cit.

opinion concerning their nature; namely, that they are globules of a more or less fluid substance which is passively subject to the forces active in the cell. The karyokinetic forces appear to cause the solution or emulsion of the nucleolar substance, which then again separates out in drops when these forces cease to be active. Such bodies can certainly not be regarded as definite organs of the cell; and it is upon the assumption that they are such that the views of both writers above quoted rest.

Furthermore, the study of spore-mother-cells of *Psilotum* and *Osmunda* has enabled me to detect, in favourably-cut microtome sections, the presence of the true centrospheres, corresponding to those described and figured by Guignard. These bodies, which Karsten has clearly overlooked, take no marked stain and are far less easy to recognize than the smallest globules of nucleolar substance. That they are to be regarded as true organs of the cell, 'kinetic centres,' there seems little doubt. My observations also throw some light on the interesting and still unsettled question of their origin. In several cases I have found them lying outside of the nucleus, though close to it, while the nuclear membrane was apparently still quite intact. Indeed, in all the plants studied they seem to be of cytoplasmic rather than of nuclear origin.

It seems also extremely probable that the granules observed by Farmer[1] in the pollen-mother-cells of *Lilium Martagon* are similar nucleolar masses to those seen by other writers; but it is equally improbable that they are 'regular and normal constituents of the cell during these stages of division.'

Incidentally my studies appear to throw some light on the nature of the body termed, ten years ago, by Strasburger, the 'paranucleolus,' and called by Zimmermann the 'sickle-stage' of the nucleolus. This crescent-shaped body is found at one margin of the nucleus, and has been supposed to represent a stage in the disappearance of the nucleolus. I have observed it chiefly in tissues of considerable thickness, and most strikingly in the pollen-sacs of *Ceratozamia*. Here, in material fixed with alcohol, almost every nucleus may show one of these bodies, and *always on the side turned away from the nearest surface of the organ*. The fixing fluid proposed by Mann often fixes *Ceratozamia* material better than alcohol, and then one finds very few

[1] Annals of Botany, Vol. VII, p. 392; Sept. 1893.

and small 'paranucleoli.' Thus it appears that this body is an artificial product, due to the unequal penetration of the fixing medium. When this penetrates a nucleus from one side it seems to carry with it certain stainable nuclear constituents, until they are stopped by the nuclear membrane, against which they heap up. In its staining relations this material shows similarity to chromatin perhaps more than to nucleolar substance, and is probably a mixture of various nuclear constituents.

J. E. HUMPHREY,
Weymouth Heights, Mass., U.S.A.

Euglenopsis: a New Alga-like Organism[1].

BY

BRADLEY MOORE DAVIS.

With Plate XIX.

THE writer first noticed specimens of this interesting organism in November, 1893, while examining some material collected in the salt-marshes of the Charles river, Cambridge, Massachusetts. It proved to be very common at that season of the year and covered stems of marsh-grass (*Spartina*) and other objects floating near the surface of quiet pools, so thickly that the surface of these bodies resembled dark green velvet in colour and texture. Mixed with it were sometimes colonies of the non-motile condition of *Cryptoglena americana*, Davis, or occasionally clusters of Diatoms, but on the whole the organism was remarkable for its habit of thickly covering its substratum to the exclusion of other forms.

The characters of this organism are such that it may not be apparent to all readers why it should be considered as a plant and not an animal, but the peculiarities of its structure and mode of growth are so interesting, and its affinities so close to certain genera usually considered as plants, that the writer feels justified in presenting the paper to botanists.

[1] Contributions from the Cryptogamic Laboratory of Harvard University, No. XXV. Prepared under the direction of Dr. W. G. Farlow.

[Annals of Botany, Vol. VIII. No. XXXII. December, 1894.]

Since the structure of this organism is peculiar and most readers are not likely to have ever met with a similar form, it will be much easier to make the description clear if the reader will first glance at Plate XIX. In Fig. 1 is shown a well-developed specimen magnified about 250 diameters. From this figure one may get a very fair idea of the habit of the plants, which are all small, the larger specimens being about one-fourth of a millimeter high. As may readily be seen, certain green cells are situated at the ends of a branching filament or stalk, and this branching filament consists of empty cell-cavities or compartments arranged in a moniliform manner.

The wall of the filament is hyaline and thin, but elastic, and so firm that it shows no tendency to collapse, and so flexible that the filament quickly regains its former shape when it has been bent. It is entirely unaffected by boiling in a strong solution of potassic hydrate, which proves that it is not of a gelatinous nature. While the wall does not give the cellulose-reaction with iodine and concentrated sulphuric acid, nevertheless its behaviour when treated with other reagents shows it to consist, if not actually of cellulose, of a substance closely related to cellulose. It dissolves in the most concentrated sulphuric acid, and can be stained slightly by iodine and readily by haematoxylin and certain anilin-dyes.

When it is stated that the cell-cavities in the lower portions of the filament are empty, it is meant that they are free from any protoplasmic matter. They also contain no bubbles of gas, and it is but reasonable to suppose that the fluid in their interior is the brackish water of the salt-marshes.

Passing now to the green cells at the ends of the branches of the filament, we find, beginning with the exterior of the cell, that the protoplasm is surrounded by a thin, hyaline, firm wall, that is similar to and continuous with the wall of the filament below it. The protoplasmic mass inside the wall (see Fig. 2 in particular, and also Figs. 8 and 12) is

inclined to be oblong in shape, from 12–20 μ long and 6–9 μ wide. It is differentiated into a nucleus, two spaces containing cell-sap, a chromatophore and a pigment-spot, which will now be described in turn.

The nucleus may readily be distinguished from the other structures of the cell without special treatment. It is situated very nearly in the centre of the cell and is held in position by a band of protoplasm that completely fills up that portion of the cell which lies between the nucleus and the sides of the wall. From this band of protoplasm, that surrounds and encloses the nucleus, a layer of protoplasm extends around the ends of the cell, on the periphery, so as to enclose two spaces containing cell-sap, one above and the other below the nucleus, as the position of the cells is normally upright.

The very large chromatophore is embedded in the peripheral layer of protoplasm. It extends as a band completely around the middle portion of the cell and usually entirely around the ends, but the latter, and particularly the upper end, are sometimes hyaline.

The cell therefore usually appears of a uniform green colour, but the shade is darker or lighter in different parts of the cell, depending upon the relative thickness of the chromatophore in those portions. There are no pyrenoids, and the small starch-grains embedded in the chromatophore are not, as far as the writer could see, arranged around any amylum-centres.

The bright red pigment-spot lies in the chromatophore usually about a third the length of the cell from the inferior end. It is very variable in size, in some cells being scarcely visible, in others very large. It reaches its greatest development in the motile stage of the plant, to be described later, where it is sometimes 1½ μ in diameter: and, when it is as large as this, a distinct granulated structure is often apparent.

The reader will have noticed the processes extending from the inferior end of the cell in Fig. 2, and also that there is often a space between the mass of protoplasm and the

partition across the filament, that separates the green cell from the empty cavity below it (see Figs. 2, 8, 12, &c.). These characters are associated with its peculiarities of growth, and will be considered after the motile condition of the plant is described.

The green cells at certain times pass into motile conditions that, from analogy to the motile stages of other Algae, may be called zoospores. Each zoospore consists of the entire mass of protoplasm of one of the green cells and is provided with four cilia. In these respects they resemble the macro-zoospores of many Algae. The change from a vegetative cell to a zoospore takes place during the night, and the latter escape from the ends of the filaments the following forenoon. They appear to be strictly non-sexual.

Some of the plants were kept for many days in aquaria, and every morning a swarm of the zoospores made their appearance at the sides of the aquaria nearest the light, thus exhibiting the phenomena of heliotropism so characteristic of zoospores of Algae. The zoospores were found early in the morning before their escape enclosed in their parent cells. Such a specimen is shown in Fig. 4. The cilia of this specimen waved slowly from side to side long before it escaped from the cell into the water.

The zoospores (see Fig. 5) are about the same size and shape as the vegetative cells, being 12–18 μ long and 6–8 μ wide, and the nucleus, chromatophore, and pigment-spot maintain the same relative positions in the former that they held in the latter. This agreement in cell-structure would be expected from the manner in which the vegetative cells change into zoospores. The pigment-spot, however, is usually larger in the zoospore than the vegetative cell, sometimes, as has been before noted, being as much as $1\frac{1}{2}$ μ wide. The four cilia, each one of which is about as long as the zoospore, are attached to the centre of that end which was inferior when the zoospore was contained in the filament. Therefore the pigment-spot is situated about one-third the length of the zoospore from the ciliated end. The non-ciliated end of the

zoospore is very apt to have less of the chromatophore and may be even hyaline.

The duration of the motile stage is but transitory. The zoospores settle down within a very few hours after their escape from the filaments, and attach themselves to some substratum preparatory to germination. When confined in a Van Tieghem cell, they immediately moved in straight lines to the edge of the drop of water nearest the light, and there attached themselves to the cover-glass. They come to rest on their ciliated ends, and the cilia may be seen to move over the substratum as the zoospores slowly revolve. The remains of the cilia are often present for some time after the zoospores have germinated. (See Figs. 6, 7, and 8.)

After the zoospore is firmly attached to the substratum, a wall is formed around it which completely encloses the protoplasmic mass in a cell that is fastened to the substratum by a disc-shaped base. In many young plants, which developed on the sides of the aquaria, the mass of protoplasm divided immediately (Figs. 9 and 10), but in most cases there resulted a curious sort of forward growth, unlike anything the writer has ever seen described among Algae.

The cell begins to elongate very soon after the zoospore has surrounded itself with a wall, and, as it lengthens, the mass of protoplasm ceases to entirely fill the cavity, but moves upwards, always remaining closely applied against the wall of the upper portion. The lower portion of the cell-cavity is therefore left entirely free of protoplasm. Within a few hours after the zoospore has attached itself to the substratum, one notices a slight space between the mass of protoplasm and the base of the cavity (see Fig. 7, a plant twelve hours old). This space grows larger as the cell elongates, until finally it becomes about equal in length to the mass of protoplasm in the upper portion of the cell.

The next change is very peculiar. The lower portion of the mass of protoplasm which before this had an undulating contour, becomes rounded off into a convex surface, and a wall is formed across the cavity. As a result there is left at the

base of the plant an empty compartment, and at the top is the green protoplasmic mass surrounded by a wall that is continuous with the wall below. Often a second cross-wall is formed, above the first and very close to it. It is as if the mass of protoplasm had contracted from below, after the first cross-wall had been formed, and developed a new wall at the point of farthest contraction. The writer never noticed more than two cross-walls above the first empty cavity, but one may often find three or even four such walls between empty cavities in upper portions of adult plants. In Fig. 1 may be seen several instances where more than two cross-walls are present between empty compartments.

The mass of protoplasm probably remains inactive for a short time after it has formed a wall separating itself from the empty space below, but very soon it begins to push upwards again, and the membrane in which it is enclosed elongates. The manner in which this elongation takes place is precisely like that in which the original wall that enclosed the zoospore lengthened. The mass of protoplasm keeps in the upper portion of the cavity, and the lower portion is left empty, and then, when the filament has so increased in length that the empty space is about equal to the protoplasmic mass, there is formed another cross-wall, and another empty compartment has been added to the filament of the plant. In Fig. 8 may be seen a specimen in which there is the empty cavity at the base of the plant, and above the green protoplasmic mass surrounded by a wall, and between the two may be seen a slight space showing that the upper cell has already begun to elongate.

The writer observed many young plants, which had developed from zoospores in his aquaria, until they consisted of a filament of several empty cell-cavities with the green cell at the free end, and there could be no doubt but that each empty cell-cavity was formed in the way just described. The filaments, therefore, increase in length by the periodic forward growth of the masses of protoplasm at their free ends. The periods of forward growth alternate with periods of rest in

which the protoplasmic mass remains quiet, at the end of the filament, long enough for one or more cross-walls to be formed at its inferior end. The periods of rest occur at such intervals of time that the cross-walls divide the filament into empty compartments, which are remarkably uniform in size and are about the same length as the masses of protoplasm.

Now that we have seen how the zoospores germinate, and understand the principal characteristics of the peculiar mode of growth, we can return to the green cells at the ends of the branches of adult plants, and consider a peculiarity of structure that was not treated in detail in the first part of the paper. This is the manner in which the inferior ends of the masses of protoplasm frequently extend downwards in the form of processes (see Fig. 2), which are closely applied against the wall of the filament. These processes contain portions of the chromatophore, but the extreme ends are usually hyaline. They are only to be found when the protoplasmic masses have left the quiet condition, and the filament is actually elongating. They are really but an exaggeration of the undulating contour of the inferior ends of the protoplasmic masses in young plants, when the latter are in the period of active growth.

The contents of a cell having these protoplasmic processes are very sensitive, and if, when living material is examined, the normal conditions are not carefully maintained, these processes are drawn in, the protoplasmic mass contracts, and the posterior end becomes rounded off. Thus when specimens were observed on a slide in salt water, this contraction of the cell-contents invariably occurred in the course of twenty to thirty minutes, apparently because the evaporation of the salt water under the cover-glass altered the density of that fluid. Similar specimens confined in a Van Tieghem cell were much less sensitive, and might be observed for a much longer time without detecting any such change, although the masses of protoplasm never continued their forward growth, and always eventually contracted into the form commonly assumed when a cross-wall is to be formed.

Let us now consider what takes place when a filament branches. The first step in this process is a division of the mass of protoplasm, which, of course, is morphologically the cell. The plane of division is usually oblique and in the general direction of the length of the cell (see Figs. 9 and 11), but sometimes the division is across the cell at right angles to the axis of the filament (see Fig. 10 and portions of Fig. 1).

When the division is oblique, the filament branches, because the two masses of protoplasm move upwards at slightly different angles. In Fig. 11 we have an excellent illustration of the oblique division of a cell, and we can readily see that the lower cell in Fig. 11, in trying to move upward, would be pushed to one side and the direction of its movement altered by the cell above. It is evident that, if the lower cell in Fig. 11 pushed out at one side and the upper cell continued to move upward, there would soon result a condition similar to Fig. 12.

When the division of a cell is transverse, that is at right angles to the long axis of the cell, the filament does not branch. Apparently the lower cell has no opportunity to move forward, and consequently remains enclosed in a compartment of the filament. The upper cell may and does continue to move upward, thus increasing the length of the filament, and one not unfrequently finds a green cell separated from the end of a filament by two or three empty cell-cavities. On the right hand side of Fig. 1 may be seen such a cell left behind by the forward growth of the filament. These cells may develope into zoospores which escape by rupturing the wall of the filament.

Occasionally after a cell-division the two daughter-cells will immediately divide again, so that as a result one finds four cells surrounded by a common cell-wall. A very usual arrangement for such a group of cells is shown in Fig. 3. In this case the plane of the first cell-division was oblique, and the plane of division of the two daughter-cells at right angles to the first. Four branches might result from such a division, if the planes of division were sufficiently oblique, so that

every cell would tend to move upward in a slightly different direction. However, the two lower cells resulting from the second division are usually so situated that they are left enclosed in compartments of the filament, while the two upper cells alone give rise to branches.

With the above we have finished our description of the anatomy of the organism. As suggested at the beginning of the paper, the position of the form, whether plant or animal, is not easy to determine, and we must give our reasons for considering it related to certain forms that are usually called plants. But we must first consider some general features of its structure and mode of growth.

The writer has used the term cell-cavity to designate the compartments of the filament in order to simplify the description, but it seems to him, that the wall of the filament does not bear the same relation to the masses of protoplasm that a cell-wall of a plant usually does to its contents. We have seen that the protoplasmic masses are very variable in shape, sometimes having processes extending from their inferior ends sometimes contracted into a compact form with a regular outline, and that the one condition may readily change into the other. This mobility of the masses of protoplasm is associated with the peculiarities of the method of growth, and indicates that the protoplasmic masses maintain a degree of independence of their enclosing filaments that is not what we expect of the contents of plant-cells as a rule.

The motile condition of the organism resembles in many ways the macrozoospores of chlorophyllaceous Algae. There are the four cilia, the pigment-spot, and the habit of coming to rest after a comparatively short period of activity, all of which characters are possessed by most zoospores. But in the non-motile condition the masses of protoplasm still retain the pigment-spot, a structure that is not generally present in the vegetative cells of Algae. The mobility of the protoplasmic masses and the fact of the pigment-spot being present in all stages of the cycle of development, suggests at once certain organisms of which *Euglena* may be mentioned as an example.

Suppose a *Euglena* in the non-motile condition, and therefore enclosed in a cyst, to push forward in a certain direction and thus to lengthen the cyst into a filament. Then let us imagine this *Euglena* to encyst itself periodically by forming a wall across the filament and occasionally to divide in such a compartment. Finally, let the *Euglena* free itself from the filament and pass into a motile condition. Such a life-history would be identical with that of our organism.

The manner in which the inferior ends of the masses of protoplasm contract and round themselves off previously to the formation of a wall across the filament certainly resembles greatly the behaviour of unicellular organisms when they are about to enter a condition of encystment. Our organism in the quiescent condition is surrounded by a wall, closely applied against the protoplasm on every side, enclosing it in what may readily be called a cyst. It is also true that the masses of protoplasm only divide when they are in the quiescent state, that is when they are enclosed in this cyst or compartment of the filament. Many unicellular organisms, the greater part of whose existence is passed in a motile condition, only divide when in the state of encystment. To the writer's mind the behaviour of our form, when passing into the quiescent state, fulfils all the conditions of the process of encystment.

It sometimes happened with the zoospores in the aquaria that, when they came to rest, the masses of protoplasm did not immediately develope into filaments, but divided in the cells which enclosed them. Such an example is shown in Fig. 9, and here one may readily see that the protoplasmic mass has collected at the top of the cell-cavity, or we may say cyst, and there divided. Two partitions across the space below the mass of protoplasm indicate that the latter paused twice before it reached its final position. It is doubtful if the zoospores under more normal conditions, that is in their natural habitat, behave in this manner, for, from the writer's observations, the tendency of this organism is to immediately develope into a filament. However, this unusual behaviour is

interesting from its resemblance to the usual habit of many organisms of dividing when they have encysted themselves after a motile stage.

The peculiarity of there being usually more than one cross-wall formed, when the masses of protoplasm enter the quiescent state, may well be accounted for by supposing that this condition comes on gradually. Whether the inferior ends of the protoplasmic masses contract from below the entire distance from the lowermost cross-wall to the last formed, or whether there is also some forward growth of the filament at the same time as the contraction, the writer cannot say. The variation in the size of the empty cavities and of the masses of protoplasm makes it difficult to draw general conclusions on this point.

However much this organism may resemble such forms as *Euglena* in the character of the cells, the peculiarities of its filament, made up of compartments, certainly give it a right to a position quite apart from any form that the writer has seen described. It is the nature of this chambered filament that has led the writer to associate the organism with certain genera that are usually considered as plants.

If this organism is to be considered as a plant, its affinities are closest with certain genera of the family Tetrasporeae, as Wille[1] considers the group. As is well known, the genera of this family differ much among themselves, and this plant has several important characters that are not to be found at all in the forms which in other respects are most nearly related to it.

The cell-structure of *Chlorangium*[2] most closely resembles this organism, and the zoospores agree in being produced singly from a vegetative cell, but there are important differences in the nature of the chromatophore, presence of

[1] Wille, in Engler and Prantl, Die naturlichen Pflanzenfamilien, Lief. 40, p. 43, 1890.

[2] Cienkowsky, Ueber Palmellaceen und einige Flagellaten (Arch. f. mikr. Anatom. B. 6, p. 421, 1870). Stein, Der Organismus der Infusionsthiere, III. Abtheilung, 1. Halfte, 1878. Wille, loc. cit.

vacuoles, number of cilia on the zoospores, and, most important of all, the stalk bearing the vegetative cells which, in *Chlorangium*, is simple in structure, and very different from the highly specialized and complex chambered filament of this plant.

The cells of *Hauckia*[1] and *Mischococcus*[2] are quite different in structure from the form we have described: the zoospores are produced several, four to eight, in a cell, and the stalks are comparatively simple in structure although more complex than that of *Chlorangium*.

The stalk of *Oocardium*[3] is chambered, but the habit of the plant is very different, and the cells do not resemble those of our plant either in the character of the chromatophore, presence of a pigment-spot, or in general shape or arrangement.

The nature of the filament with its compartments, resulting from the curious manner of growth, together with the structure and mode of formation of the zoospores, appear to the writer to be the most important characters of this plant, and these peculiarities make it desirable to describe it as—

Euglenopsis, nov. genus[4].

Plants filamentous, branching above; filaments formed of compartments, those below empty, the terminal containing green cells; cells with a nucleus, a peripheric band-shaped grass-green chromatophore, and a red pigment-spot. Reproduction by zoospores, four-ciliate, otherwise agreeing with the cells in structure; sexual reproduction unknown.

Euglenopsis subsalsa, nov. species.

Filaments moniliform, when mature about $\frac{1}{4}$ mm. long,

[1] Borzi, Hauckia, nuova Palmellacea (Nuov. Giorn. bot. Italiano, vol. xii. 1880). Wille, loc. cit.

[2] Nägeli, Gattungen einzelliger Algen, p. 80, 1849. Wille, loc. cit.

[3] Nägeli, loc. cit., p. 74. Wille, loc. cit.

[4] In the diagnosis of the genus and species we have used the term *compartment* to designate the cell-cavities of the filaments, and the word *cell* has been reserved for the masses of protoplasm contained in the compartments at the ends of the filaments.

first simple then di-tri-chotomously branching; cells oblong, 12–20 μ long, 6–9 μ wide; nucleus centrally located; chromatophore bright green, usually extending entirely around the periphery of the cell; pyrenoids absent; pigment-spot bright red, situated near posterior third of cell, very variable in size; compartments of filament about the same size as the cells, separated from each other by 1–4 cross-walls; wall of filament thin, hyaline; zoospores same size as vegetative cells; cilia about as long as the zoospores. situated at the end nearest the pigment-spot.

Habitat.—Salt-marshes of the Charles river, Cambridge, Mass. The plants grow very close together, forming a green velvet on the sides of marsh-grass and other objects on or near the surface of the water. Autumn.

EXPLANATION OF FIGURES IN PLATE XIX.

Illustrating Mr. Davis' paper on *Euglenopsis*.

All figures drawn from nature and sketched with an Abbe camera. Fig. 1 magnified 250 diameters, all others 750 diameters.

Fig. 1. Specimen showing habit of plant. × 250.

Fig. 2. Tip of branch, showing terminal cell at a time when it is growing forward, illustrates the appearance of the protoplasmic processes at the posterior end of the cell; three cross-walls are between the cell and the empty cell-cavity just below it.

Fig. 3. End of a branch illustrating a case in which four cells have been derived from one. The first division of the original cell was oblique in a longitudinal direction, and then each of the two cells thus formed divided again in a plane at right angles to the plane of the first division.

Fig. 4. A zoospore at the end of a branch still enclosed in the terminal compartment, the cilia were moving slowly when the drawing was made. Four cross-walls between the terminal compartment and the one adjacent to it.

Fig. 5. Zoospore killed with Flemming's fluid.

Fig. 6. Zoospore just settled down preliminary to germinating; drawn from a specimen in a Van Tieghem cell.

Fig. 7. Young plant twelve hours old.

Fig. 8. Young plant thirty-six hours old, with one empty compartment.

Fig. 9. Young plant thirty-six hours old; cell has divided obliquely after forming two cross-walls at the base.

Fig. 10. Young plant thirty-six hours old; cell has divided at right angles to the long axis of the plant.

Fig. 11. Plant thirty-six hours old with one empty compartment.

Fig. 12. Plant about two and a half days old.

Contributions to the Life-History of Notothylas.

BY

DAVID M. MOTTIER,

Associate Professor of Botany, Indiana University, Bloomington, U.S.A.

With Plates XX and XXI.

THE genus *Notothylas* is represented in Indiana by two species, *N. orbicularis*, Sulliv. and *N. melanospora*, Sulliv. The former, according to Gray's Manual[1], is pretty widely distributed over the Eastern United States.

Notothylas orbicularis, the species with which we are particularly concerned here, grows abundantly upon damp, shady ground where the moisture is tolerably constant during the warmer parts of the year. Not infrequently were specimens found side by side with *Anthoceros*, the lobes of the thallus sometimes overlapping, so that it was often difficult to distinguish one from the other. However, *Notothylas* is of a paler green, the lobes of the thallus smaller and much more irregular than those of *Anthoceros*.

This year, fruiting specimens were collected during the latter part of July and up to August 10, but these are more abundant from October until the plants are frozen in November, when almost every specimen appears to bear

[1] Revised Edition, p. 727.

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sporogonia. By gathering specimens earlier in the season one is sure to find both sex-organs and sporogonia in different stages of development on the same semicircular or circular expansion of thallus.

According to the views of Leitgeb [1] and Goebel [2], *Notothylas* is regarded as representing a transition from *Anthoceros* to the Jungermannieae. This view is based mainly upon the structure of the sporogonium, particularly the limited growth of its capsule and the nature and origin of the columella.

Hofmeister has also made careful and extended observations upon the Hepaticae, but unfortunately his works are not accessible.

Goebel [2] states that the capsule of *Notothylas* either has not a columella, or has one which is only a secondary differentiation inside the spore-chamber. In another place [3] the same author says that there are species of *Notothylas* which possess a columella similar to that of *Anthoceros*, but of its origin nothing is known; that, in fact, it is uncertain whether this arises, as in *Anthoceros*, with the archesporium though independently of it, or whether it is a product of a gradual differentiation in the spore-chamber.

For a detailed knowledge of these Liver-worts we are indebted to Leitgeb, who, in his admirable work *Untersuchungen über die Lebermoose*, has published the results of careful and extensive investigations.

Four species of *Notothylas* were examined by this author, *N. fertilis*, *N. valvata*, *N. Breutellii*, and *N. melanospora*.

Leitgeb [4] states that in some species of *Notothylas*, there occur capsules, which, in regard to the size (Mächtigkeit) of the columella and the difference of its cells from the other sterile cells of the spore-chamber, do not differ from the capsules of *Anthoceros*; and it may be possible, though not probable, that these capsules, in respect to the origin of their

[1] Untersuchungen über die Lebermoose, Heft V, 1879.
[2] Outlines of Special Morphology, p. 158, English translation (1887).
[3] Schenk in Handbuch der Botanik, Band II, p. 357 (1882).
[4] Loc. cit., Heft V, p. 7.

columella and spore-forming layer, agree with *Anthoceros*. That, moreover, in all species one finds capsules in which a columella is present, but where the cells of the columella are formed throughout similarly to the other sterile cells of the capsule, and may be very easily separated from one another. The columella of such capsules is not developed, as in *Anthoceros*, independently of the spore-forming layer, but arises as a secondary differentiation within the spore-chamber, and in this respect agrees with the columella of the Moss-capsule. Again, in his conclusions he says: 'In many (perhaps all?) species of the genus *Notothylas* there are capsules that possess no columella, and the examination of half-ripe sporogonia shows that this is not due to the eventual separation of the cells, but that no columella is ever differentiated.' These conclusions were drawn from a study of the four species mentioned above. In *N. fertilis*, as many capsules were found without the columella as with it, and in no case did the columella extend farther up than the middle of the capsule. The other species possess a columella.

According to Gottsche [1], *N. fertilis* is identical with *N. valvata*, and there is reason to believe that *N. valvata* is the same as *N. orbicularis* [2], the species here under consideration.

The question to be answered then is what is the origin of the columella? Does it arise as in *Anthoceros*?

This work, the results of which are set forth in the following pages, was taken up at the suggestion of Dr. Douglas H. Campbell of the Leland Stanford Jr. University.

It may be well first to give a brief outline of the method used, as the results will thereby be more fully understood and appreciated. All specimens were fixed in chromic acid (1 per cent. aqueous solution), remaining in the fluid about two hours; then, after washing repeatedly in water for about two days, they were brought gradually into 70 per cent. alcohol, where they remained until wanted. In this way all

[1] Leitgeb, loc. cit., Heft V, p. 40, footnote.
[2] Gray's Manual, revised edition, p. 727.

traces of the acid are removed, a condition so necessary when it is desirable to stain with alum-cochineal. They were then stained *in toto* with alum-cochineal, dehydrated, brought gradually into a solution of turpentine and paraffin, imbedded in paraffin, and sectioned on a Minot-microtome. The sections were counter-stained on the slide with Bismarck-brown dissolved in 70 per cent. alcohol, and mounted in Canada-balsam. The nucleus and parts of the protoplasm are stained by the cochineal, and the cell-walls by the Bismarck-brown. In this way all details are clearly and beautifully brought out.

With the view of contributing something toward the solution of the problem stated above, a study of the development of the sporogonium, especially the earlier stages, was made, together with that of the antheridia, to determine, if possible, whether the latter arise from an epidermal or a sub-epidermal cell.

For the purpose of comparison, a similar study of *Anthoceros* was carried on with that of *Notothylas*.

The first divisions of the embryo correspond to those which regularly follow in all known Liver-worts. The fertilized egg is divided into an upper and a lower cell by the basal wall, which is at right angles to the long axis of the archegonium, the former becoming the capsule and the latter the foot of the sporogonium. Now follow two walls in rapid succession at right angles to the primary wall and to each other, thus dividing the embryo into eight cells disposed as the octants of a sphere. The exact order in which the two latter walls were formed was not determined. Fig. 1 represents three successive vertical sections of such an embryo, which include the whole of it. The eight nuclei were so situated that four came in the first section (*a*) and four in the third (*c*), while in the middle one (*b*) there were no nuclei. Serial sections of a similar embryo, at right angles to the archegonial axis, revealed similar structures.

The embryo, however, is usually more oval in shape (Fig. 16).

The four lower octants develop into the foot, as described by Leitgeb[1].

The four upper octants are now divided by transverse walls into two tiers of cells disposed as quadrants. It is barely possible that occasionally three tiers are formed (Fig. 2). Each of these cells next divides by a periclinal wall into an inner and a peripheral cell. The inner cells become the columella, while the peripheral cells give rise to the archesporium and the wall of the capsule, precisely as in *Anthoceros*. This will be readily seen by a comparison of Fig. 6 *b* with Fig. 15, which are transverse sections of sporogonia of the same age respectively. The differentiation of the archesporium from the peripheral cells appears to begin at the apex, proceeding toward the base (Fig. 3).

In *Notothylas*, however, a number of difficulties are met with in the demonstration of these facts. Cell-division does not follow with as great regularity as in *Anthoceros*, and the difference between the archesporial cells and those of the columella is not so pronounced. Here the cells of the archesporium, in sporogonia about the size of those in Figs. 4 and 5, are only a little richer in protoplasm than those of the columella, requiring careful staining and exactly straight sections in order to make out the distinction. Considerable difficulty was experienced in orienting specimens to get perfectly straight longitudinal sections through the sporogonia, from the fact that the sporogonia do not stand perpendicular to the surface of the thallus, but slightly inclined forward; and that the small lobes of the thallus do not invariably lie flat, but they are more or less tipped by the wrinkling or wave-like folding of the thallus in its growth. Besides, all sporogonia are disposed radially on the circular or semicircular thallus, no two lying exactly parallel. Several preparations were obtained, however, in which the sections passed exactly straight through the sporogonium with the above results (Figs. 3 and 4).

In all cases observed it was evident that the archesporial

[1] Loc. cit. p. 49.

cells over the apex of the columella multiplied by tangential divisions, so that this region consisted of a mass of archesporial cells (Figs. 4 and 5), instead of a single layer, as in *Anthoceros*.

Transverse sections reveal the same structures as those seen in longitudinal sections. Fig. 6, *a*, *b*, and *c*, represent cross-sections of a sporogonium of about the same stage in development as Fig. 5, taken at *a–a*, *b–b*, and *c–c* respectively: Figs. 7 to 14, inclusive, represent successive cross-sections, including all of a similar sporogonium above the foot. In Fig. 7 the centre is occupied by four cells, the columella, surrounded by a peripheral row. In Figs. 8, 9, 10 the archesporium is seen, in addition to the other two regions. There can be no question as to its origin. These sections are not unlike Fig. 15, save in the latter the cell-divisions take place with diagrammatic regularity. In Fig. 11 the section passes through the extreme tip of the columella, of which three cells are shown. Figs. 12, 13, 14 embrace the remainder of the sporogonium above the columella.

The sporogonium of *Notothylas* undergoes intercalary growth, just as in *Anthoceros*, and the archesporium and columella extend quite to the foot (Fig. 19). In one case observed (Fig. 19), the cells of the foot were quite regular; they had not grown into the irregular tubes which is almost invariably the case. The intercalary growth is, of course, of comparatively short duration, but as long as it lasts there cannot properly be said to be a stalk in the sense of that term as used in the Jungermannieae. It is only when the capsule is mature that the cells immediately connecting foot and capsule elongate and finally break away from the foot, thereby severing all organic connexion with the latter.

As is stated by Leitgeb[1], the size of the sporogonium is no proof of its age. Not infrequently does one find on the same thallus small sporogonia with others very much larger. Many of these smaller sporogonia, which appear under the dissecting-microscope to be younger stages, have in their apex spore-

[1] Loc. cit.

mother-cells, and sometimes spore-tetrads and ripe spores (Fig. 18). The columella is always present, but much smaller than those of the larger sporogonia (Fig. 18), and its cells are distinguished from those of the archesporium with some difficulty, especially if the section is not straight and more deeply stained. In the case figured in Fig. 18, the cells of the columella were little unlike those of the archesporium in regard to the contents of the cells. In all capsules examined, a columella was found extending up through the centre of the capsule nearly to the apex, varying in size with that of the capsule. From the columella layers of sterile cells extend radially to the capsule-wall, dividing the spore-chamber into more or less irregular cavities in which lie, proceeding downward from the apex, spores, spore-tetrads and spore-mother-cells. By making rather thick longitudinal sections through a nearly mature capsule, and freeing the spores by tapping gently with the end of a camel's-hair brush, one readily finds the columella with few sterile cells and spore-tetrads adhering (Fig. 21). The sterile cells fall out with the spores, and may be found scattered among them in the water. They are derived from the archesporium (Fig. 17). The capsule from which Fig. 17 was taken contained spores in its upper two-thirds: the columella is quite large, and the archesporium extends quite to the boundary between foot and capsule, where, in this case, the cells were just beginning to elongate preparatory to the separation of the capsule. In all robust and well-fed sporogonia (and many were examined) the cells of the archesporium were rich in protoplasm, staining deeply with alum-cochineal, and forming a sharp contrast to those of columella and capsule-wall. The structure of the mature capsule in the Anthoceroteae has been carefully described by Leitgeb[1], so that further details need not be given here. The process of division in the spore-mother-cells, as far as observation went, agrees with that given by Strasburger for *Anthoceros*[2].

[1] Loc. cit.

[2] Zellbildung und Zelltheilung. Third edition, p. 158, &c. (1880).

As was stated in the preceding pages, there is marked variation in the size of the sporogonia of *Notothylas*. Fig. 20 represents a longitudinal section of a young sporogonium, differing considerably from all others observed—of the same size—especially in regard to the foot, which seems to have developed very little. From the fact that it was in a very slender lobe of the thallus, and that its cells with small nuclei were relatively poor in protoplasm, it is possible that we have here a starved specimen. No other similar case was observed.

From the history of development here observed it is evident that the columella of *Notothylas* (wherever it occurs) is of primary origin, and this, together with other parts of the sporogonium, agrees closely with *Anthoceros*. There is, therefore, a closer relationship existing between this and the other genera of the Anthoceroteae than has been previously supposed.

The Archegonium.

The development of the archegonium was found to agree with the account of Janczewski and Leitgeb[1]. My preparations, however, showed the various stages very beautifully, and a few of these will be figured for the purpose of comparison (Figs. 22–24). In the mature organ, however, slight differences were noted. In both genera, the neck-cells of the archegonium are quite inseparable from those of the thallus; but in younger stages, in *Notothylas*, some of the neck-cells could be easily distinguished from the adjacent cells of the thallus by their denser contents. Occasionally the neck projected slightly above the surface of the thallus. In *Notothylas*, the neck-canal-cells were always fewer in number than in *Anthoceros*, amounting to three, not including the cap (Deckelzelle of Leitgeb), in the former (Fig. 22); and five or six in the latter, but not exceeding six in any case observed. In this respect, *Notothylas* resembles more closely certain

[1] Loc. cit. pp. 20, 21.

eusporangiate Ferns as *Angiopteris* [1]. Leitgeb [2] states that the lateral walls of the neck-canal-cells during the process of growth become very strongly thickened, and this thickening extends to parts of the cap (Deckelchen) and even to the central cell; that they are finally transformed into a gelatinous layer, while the cross-walls remaining very thin are completely dissolved. It seems to me that the lateral walls are not thickened at all, but the phenomenon is due to the presence of a swelling mucilaginous substance derived from the ectoplasm of the cells (Figs. 22, 23). This mucilaginous formation begins, it is true, at the ventral canal-cell, and proceeds upward. Very frequently, however, the egg is surrounded by a mucilaginous formation staining deeply with Bismarck-brown, while that in the neck stains only very slightly. The ventral canal-cell is usually as large as the egg in the mature organ (Figs. 22, 23).

In one case observed the large cap-cells projected considerably above the surface of the thallus (Fig. 24).

The Antheridium.

In regard to the antheridium, I am forced to the conclusion of Waldner and Leitgeb [3], that this organ arises from a hypodermal cell, and that, if the mother-cell be, in any case, epidermal and become grown over later by the surrounding tissue, this process takes place at a time when the mother-cell cannot be distinguished as such.

Special care was taken in working out this particular detail, and in all the youngest stages recognizable, the mother-cell was found beneath the epidermis. Fig. 25 shows two mother-cells formed by the longitudinal division of an original mother-cell. It will be seen that the formation of the cavity in which the antheridia lie has just set in. Figs. 26 and 27 show two older stages. A further detailed account of the growth of the antheridium would be superfluous here. The

[1] Farmer, Annals of Botany, Vol. vi, pp. 265-270, Oct. 1892.
[2] Loc. cit. p. 21.
[3] Loc. cit. p. 17.

antheridial cavity is roofed over by a layer of two, occasionally three, cells in thickness. As the antheridia approach maturity they gradually burst through this roof, the cells of which separate near the centre and turn back, forming the edge of the cup-shaped cavity in which antheridia of different ages stand side by side. The cells of the roof, at the time of opening, appear more rounded, with thinner walls, so that it seems that a dissolving agent acts with the mechanical pressure of the antheridia. Very frequently there are much younger antheridia in the same cavity with the mature ones, a fact which seems to give further evidence of an endogenous origin.

The development of the antheridium of *Notothylas* is precisely like that of *Anthoceros*.

SUMMARY.

The results of the foregoing statements may be summed up as follows:

1. The capsules of *Notothylas orbicularis*, Sulliv. possess a columella varying in size with that of the capsule.

2. The columella originates, as in *Anthoceros*, primarily in the young sporogonium with the archesporium, and independently of it, and consequently it is not a secondary differentiation within the spore-chamber.

3. The archegonium of *Notothylas* resembles more closely that of the eusporangiate Ferns than does the archegonium of *Anthoceros*.

4. The antheridium arises from a hypodermal cell, a process occurring nowhere else in the whole group of Bryophytes.

I desire to acknowledge my indebtedness to Dr. L. M. Underwood for the use of important and necessary literature.

EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Mr. Mottier's paper on *Notothylas*.

Fig. 1. The three successive longitudinal sections of an eight-celled embryo of *Notothylas orbicularis*; the arrow pointing toward the fore edge of the thallus. × 520.

Fig. 2. Longitudinal section of an older embryo. × 520.

Fig. 3. Longitudinal section of a young sporogonium of *Notothylas*, showing columella and four archesporial cells arching over it; *I–I*, basal wall. × 372.

Fig. 4. Longitudinal section of an older sporogonium of *Notothylas*; the cells of the archesporium with contents indicated. Those just over the apex of columella have divided by tangential walls. × 372.

Fig. 5. Similar to Fig. 4, but a little older. × 372.

Fig. 6. Transverse sections of sporogonium of about the same age as 5; *a* taken at *a–a* (5); *b* at *b–b*, archesporium indicated; *c* at *c–c*. × 372.

Figs. 7–14. All the successive cross-sections of a sporogonium of *Notothylas* a little younger than Fig. 5, from the basal wall upwards. × 372.

Fig. 15. Transverse section of a young sporogonium of *Anthoceros*, showing the four central cells, the columella surrounded by the archesporium, and then the peripheral row. × 372.

Fig. 16. Longitudinal section of an eight-celled embryo of *Notothylas* with the surrounding cells of the thallus. × 372.

Fig. 17. Longitudinal section, showing half of the lower part of a nearly mature capsule of *Notothylas*, in which the origin of the sterile cells from the archesporium is evident. At the boundary between foot and capsule the cells are becoming slightly elongated preparatory to the separation of the capsule. × 250.

Fig. 18. Longitudinal section of a small sporogonium of *Notothylas* with a portion of the foot omitted. Several spore-mother-cells lying loosely in the spore-chamber are rounded off and almost ready to divide. The columella is small, but unquestionably present; the archesporium extends to the region of the basal wall. × 250.

Fig. 19. Longitudinal section of sporogonium of *Notothylas*, showing foot and lower portion of capsule. The cells of the foot are quite regular; the columella and archesporium extend entirely to the foot. × 350.

Fig. 20. Longitudinal section of an apparently starved embryo of *Notothylas*, from a very slender lobe of the thallus. × 372.

Fig. 21. Portion of a columella to which adhere several sterile cells and three spore-tetrads. Fresh preparation in dilute glycerine. × 94.

Fig. 22. Longitudinal section of an archegonium of *Notothylas* shortly before opening. The contents of the cells immediately surrounding the archegonium are indicated. × 750.

Fig. 23. Longitudinal section of an archegonium of *Anthoceros*; the wall between egg-cell and ventral canal-cell has disappeared; the rounded contents of these two cells are surrounded by gelatinous substance, a condition almost invariably present when the organ is ready to open. ×520.

Fig. 24. Similar to Fig. 23, but with high projecting cap-cells. ×520.

Figs. 25–27. Longitudinal vertical sections representing three early stages in the development of the antheridium of *Anthoceros*. In Fig. 25 may be seen two antheridium-mother-cells formed by longitudinal division of a primary cell. ×520. Figs. 26 and 27, ×372.

The Cause and Conditions of Lysigenous Cavity-formation.

BY

FREDERICK C. NEWCOMBE, Ph.D.

Assistant Professor of Botany in the University of Michigan.

IT has long been known that in the formation of lysigenous cavities during primary extension in plants there is a tearing apart of cells due to tension between tissues, while in plants in which lysigenous cavities arise subsequently to primary growth there is generally a collapse, but little or no tearing of cells. The relation of these two processes of cavity-formation to one another has however, so far as I know, not been the subject of consideration. Moreover the extent to which this tearing during primary growth is a determining factor in the duration of the life-period of the cells concerned, is a matter which has not been determined.

The following pages will thus treat of these two questions:—

1. What are the conditions of lysigenous cavity-formation during and subsequently to primary growth?

2. Would cells that are destroyed by tearing during primary growth live much longer if the tearing did not take place?

In this study the following plants have been used for observation and experiment:—

Allium Cepa, L.	*Dahlia variabilis*, W.
Althaea taurinensis, DC.	*Equisetum limosum*, L.
Archangelica sativa, Mill.	*Eryngium planum*, L.
Caltha palustris, L.	*Forsythia viridissima*, Lindl.
Cucurbita Pepo, L.	*Helianthus tuberosus*, L.

[Annals of Botany, Vol. VIII. No. XXXII. December, 1894.]

Juglans nigra, L.	*Sambucus nigra*, L.
Juncus effusus, L.	*Silene viridiflora*, L.
Lamium garganicum, L.	*Taraxacum Dens-leonis*, Desf.
Melianthus major, L.	*Triticum repens*, L.
Myrrhis odorata, Scop.	*Urtica dioica*, L.
Pterocarya fraxinifolia, Nutt.	*Vicia Faba*, L.
Ricinus communis, L.	*Zea Mais*, L.

This work was begun in the Botanical Laboratory of the University of Leipzig under the direction of Professor Pfeffer, to whom the author feels himself greatly indebted, and was completed in the University of Michigan.

1. Lysigenous Cavity-formation during Primary Growth.

As far as observation has extended, all cavity-formation during primary growth is in its beginning schizogenous. But this splitting apart of cells before collapse of cells ensues may be on the one hand very extensive, while on the other hand it may go no farther than the formation of small intercellular spaces. The process has been described for several plants by Frank [1], Trécul [2], and others, and a general review of the matter is given by De Bary [3]. The cause for the appearance of these schizogenetic clefts is to be found in the rapid extension of the peripheral zones of tissue opposed to the more slowly extending or wholly non-extending tissue in the localities where the clefts arise.

Using for illustration only those plants which have been the subjects of my own observation, the leaves of *Allium Cepa*, the peduncle of *Taraxacum Dens-leonis*, the upper part of the stem of *Vicia Faba*, and the stem of *Caltha palustris*, may be cited as examples in which the splitting apart of cells during primary extension gives rise to central cavities of considerable size before any cells die.

[1] Frank, Beitr. zur Pflanzenphysiologie. Leipzig, 1868, p. 145.
[2] Trécul, Ann. Sci. Nat. 4e sér. I, p. 166.
[3] De Bary, Vergleichende Anatomie, §§ 51, 52.

In *Allium Cepa*, while the leaf retains in cross-section the semilunar form, the parenchymatous network of central cells is wholly living. When the leaf however begins to assume its inflated form, accompanied by the great growth in size of the peripheral cells, the central network is broken by the splitting of cell-walls, so that there arise plates of cells projecting freely into the schizogenous cavity and attached along only one border of the plate. These cells, however, at this time contain a good supply of protoplasm and still live for a period, but at last collapse, adding thus lysigenously to the size of the already existing schizogenous cavity.

What has been stated for *Allium* is true in general also for a great many other plants, especially for the peduncle of *Taraxacum Dens-leonis*, the stem of *Caltha palustris*, and the upper internodes of *Vicia Faba*. In all of these, the cells where the cavity is forming suffer a considerable separation into plates or strands, so that a large part of the cell-surface is exposed to the space of the cleft before any cells die.

But this procedure is not the one found in all plant-organs that form lysigenous cavities during primary growth. All but the lowermost internodes of the stems of *Cucurbita Pepo*, *Dahlia variabilis*, *Archangelica sativa*, *Myrrhis odorata*, *Melianthus major*, *Ricinus communis*, and many other plants, proceed no farther in the schizogenous formation of the central cavity than to produce ordinary intercellular spaces, or at most to increase the size of such spaces to very small clefts, before the collapse of cells begins. Thus, for instance, though during primary growth the schizogenous formation of cavity in the pith of *Cucurbita Pepo* and *Melianthus major* is at first relatively small, the cavity becomes very large by the collapse of a few central cells occurring at a relatively earlier period than in the first group of plants cited, and by the subsequent great extension of the cells lying just outside those that collapse.

Between these two extremes, in the one of which the schizogenous process is of large extent, and in the other goes at first but little farther than the formation of ordinary inter-

cellular spaces, there are all gradations shown by different plants. The essential difference of the two processes resides in the unlike development of the pith. In the first case the central and peripheral parts of the pith mature at about the same time, so that the schizogenous cleft is large and is followed by the rapid collapse of cells throughout. In the second case the central part of the pith matures first, while the peripheral part continues to grow, so that the first schizogenous clefts, though small, are followed by the collapse of the few surrounding cells, and the cavity increases in size by the continued outward movement of the peripheral zone of pith and by the collapse of a few cells bounding the already existing cavity. Considered *in toto*, the schizogenous factor may be much larger in this latter case than in the former, though the initial clefts are much smaller in the latter.

2. Lysigenous Cavity-formation subsequent to Primary Extension.

Although with the cessation of primary extension the increase of tension between the pith and the more peripheral tissues, due to displacement of the peripheral zones, goes no farther, there are cases found in which cavities, though not appearing till after primary growth is complete, nevertheless begin schizogenously. In illustration of this statement may be mentioned the cavities in the pith of *Althaea taurinensis*, *Silene viridiflora*, and *Eryngium planum*. The cavities in these plants, due mostly to the collapse of tissue-elements, show themselves in their initial stage as small clefts between living cells. In the particular plants just named the cavities appear soon after the completion of primary growth; the schizogenous clefts are, however, formed during primary growth, but the adjoining cells live for some time afterward, that is, till secondary growth has begun.

Schizogenous clefts may, however, appear in the pith a long time after the surrounding zones of tissue have ceased to travel outward from the centre of the stem. We have only to

think of the pith-cells as diminishing the amount of their turgor while the outward pull of the more peripheral zone remains constant, to understand how, by the consequent inclination of the less turgid cells to decrease in size, the tension between such tissues is increased, and ensuing splits may follow before any cells die. In this manner are to be explained the clefts found in the pith of the lower internodes of many plants, which mark the beginning of cavity-formation a considerable period after primary extension has ended.

In by far the largest number of plants in which lysigenous cavities appear in the pith after primary growth has ended, there is a collapse of cells before their separation by more than ordinary intercellular spaces. This is the case with the rhizome of *Triticum repens*, the cavity not being present for some weeks after the full diameter of the rhizome has been attained. The cells collapse and may split apart in so doing. The lower internodes of *Lamium garganicum*, *Urtica dioica*, *Dahlia variabilis*, *Archangelica sativa*, *Vicia Faba*, *Ricinus communis*, and many other plants, form their cavities in the pith by the shrinking and collapse of cells, without showing previous separation of cells.

In the foregoing examples there is a continuous cavity formed through each internode. Another group of plants in which the cavity is interrupted by diaphragms, is represented by *Juglans*, *Pterocarya*, and *Forsythia*. The formation of these diaphragms has been studied and described by Kassner[1]. The cells of the pith, none of which die for weeks after secondary growth has begun, begin to contract and separate in horizontal planes some distance from one another. Both the radial and longitudinal pull on the pith-cells is thus relieved, partially at least, so that the cells forming a horizontal plate midway between the planes where separation and collapse of cells has begun, are not pulled apart. Cells both above and below this plate of tissue are drawn nearer and nearer to it, till at last there remains only a comparatively

[1] Kassner, Ueber das Mark einiger Holzpflanzen. Inaug. Diss. Breslau, 1884.

thin diaphragm of dead, shrivelled cells with a rather wide cavity on each side.

From this condition of the pith, in which some cells retain their position, we can pass on to that in *Sambucus* and *Helianthus*, in which all of the cells, though dead, retain their position, and the dead tissue has in it only the ordinary intercellular spaces. In these two plants, as is well known, the pith dies during the first season's growth, but not till a wide zone of secondary formation has arisen. Details need not be given here of various other plants in which the death of the pith takes place one or more years after the first season, and it need only be mentioned that in such cases the pith sometimes collapses, while at other times the cell-skeletons retain their primary position. The work of Gris[1] may be consulted for the age at which the pith of various plants dies.

3. Lysigenous Cavity-formation either previous or subsequent to the Cessation of Primary Growth.

It has been shown that nearly the same appearances accompany the formation of cavity during and subsequently to primary extension. In both stages of growth the cell-walls may be split apart, and intercellular clefts be formed, before any cells die; in both, the collapse of cells may begin before the cells are separated by large clefts. It remains to be stated that there are plants of the same species in which, in corresponding internodes, the cavity is in one individual formed during primary growth, while in another it appears at a longer or shorter time after the cessation of primary growth.

Urtica dioica and *Dahlia variabilis* verify the truth of the last statement. A strong plant of the former species exposes above the ground but five or six internodes before a cavity appears in the third internode, elongation being there incomplete. Slender plants, however, may grow to a height of twelve internodes above the soil and still show no cavity

[1] Gris, Sur la moelle des plantes ligneuses. Ann. Sci. Nat. 5e sér. XIV.

anywhere, though the lowest half-dozen internodes are fully elongated. In the average plant of *Dahlia* the cavity in the pith will be found present in all the internodes except the one nearest the ground before elongation has ended. Slender plants grow to a height of seven or more internodes, with the lowest five fully elongated, before a cavity appears.

The histological differences attending the formation of such cavities in corresponding internodes before and subsequently to primary extension are not great. When the cavity appears while radial displacement of the vascular ring is progressing, the clefts between the cells become larger before cells collapse than in the other case, where primary extension has ended before the cavity forms. Moreover, in cross-section the cells of the pith are easily seen to be smaller in those slender stems in which the pith lives on into the period of secondary formation, than in the individuals in which it dies earlier. There is also apparent in the vascular and cortical zones of the thick stems a greater tangential expansion relatively to the size of the pith than in the slender stems. In other words, the primary radial and tangential extension of cortex, vascular zone, and pith have, in the slender plants, more nearly coincided in time than in the thick ones. The length of the internodes, however, is as great in the slender as in the stronger plants.

When the foregoing facts are properly arranged it will be seen, I believe, that the formation of cavity during primary extension is to be traced ultimately to the same cause as the formation of cavity or the death of the pith subsequently to the cessation of primary extension, that is, to the fact that the cells concerned have reached the stage where, without the pull of the more peripheral tissues, they would soon die. That, however, the life-period of such cells would be slightly prolonged did this forcible tearing not occur will appear from what follows.

We have, then, a series in the formation of cavity, the one extreme of which falls in the period of primary extension and is represented by the axial tissue of the leaves of *Allium Cepa* and of the stem of *Dahlia*, while the other extreme falls in the

period of secondary growth and is represented by the pith of *Juglans* and of *Sambucus*.

The immediate and apparent cause of the formation of cavity in such cases as those cited for primary growth, lies in the inability of the central mass of tissue to keep pace in growth with the more peripheral zones. If the peripheral zones continue to expand for a considerable period after the central mass has ceased growing, or if the peripheral part grows for a considerable time much more rapidly than the central part can extend, there will be formed a large central cavity during primary growth. If, however, the peripheral tissue ends its primary extension soon after that of the central mass, the cavity formed during primary growth will be small, but the cavity will subsequently continue to enlarge by the lysigenous process.

The latter of the two cases just cited is that of some of the lower internodes of many plants, including *Vicia Faba*, *Dahlia*, and *Ricinus*, and passes insensibly into the condition in which the primary extension of the pith persists to the completion of primary extension in vascular zone and cortex. In the latter case the cavity-formation may begin soon after the ending of primary extension, or the pith may live on for weeks or years. The fact that in some plants, as pointed out for *Urtica dioica* and *Dahlia*, the cavity appears before or subsequently to the completion of primary growth according to the amount of primary extension of vascular zone and cortex relative to that of the pith, indicates that the life of the pith-cells is shortened by the tearing apart to which they are subjected in the one case, and indicates in the other case that the cells would not live much longer if not subjected to the tearing. But since, as has been pointed out by Kraus[1], the stretching of cells due to turgor increases as they pass from the embryonal condition and decreases as they assume their permanent condition, and since the parenchyma, with thin walls of cellulose, such as generally makes up the pith, must contract when the force of

[1] Kraus, Die Gewebespannung des Stammes und ihre Folgen. Bot. Zeitung, 1867, p. 105.

turgor is withdrawn, it follows that in those stems in which the extension of the pith, or, better, the positive tension of the pith, ceases with the end of primary growth, there must be a contraction of the pith as the latter loses more and more of its turgor. When the negative tension thus called forth is considerable, schizogenous clefts may precede the collapse of cells.

In those plants like *Sambucus* and *Helianthus tuberosus,* in which the pith dies during secondary growth without collapsing, it is probable, as found true by Kraus for the two plants mentioned, that the pith is in the condition of positive tension for some time after the beginning of secondary growth. When the cells lose their turgor, the force of contraction is not sufficient to separate them.

4. Effect of Prevention of Tension.

Many years ago Sachs[1] found that leaves of *Allium Cepa* grown in the dark were not hollow; he did not, however, describe the difference in the histology of normal and etiolated leaves. It is easy to understand the immediate cause of the normal formation of the cavity when observing that the etiolated leaves have in cross-section a semilunar shape, with peripheral cells slightly oblong but not of the well-known H-palisade form, while the inflation of the leaf goes hand in hand with the rapid growth and consequent tangential enlargement of these peripheral cells. In this case then, when we prevent this inflation of the leaf by growing it in the dark, we prolong the life of the central parenchymatous cells. Many individuals have been thus grown in the dark, and leaves a foot long produced altogether of living cells, whereas normally the leaf becomes hollow at a distance of five to eight centimetres from the point of its emergence from the bulb.

If zones of these leaves are etiolated by opaque wrappings while the rest of the leaf is exposed to the light, the leaf will

[1] Sachs, Ueber den Einfluss des Tageslichtes auf Neubildung und Entfaltung verschiedener Pflanzenorgane. Bot. Zeitung, 1863, Beilage.

become hollow through the etiolated zone, because there is in such segments an expansion great enough to tear the central cells. This expansion is to be traced to the effect of the expansion in the adjacent inflated parts of the leaf. If however, instead of a yielding opaque band, gypsum [1] is used to enclose zones of the leaf, the whole tissue of the enclosed part will remain alive, though the cavity will exist as normally both above and below the limits of the cast. By this process the central mass of cells has been kept alive within the cast eleven or twelve days after it had died outside of the limits of the cast.

Similar results have been obtained by enclosing the aerial shoots of *Juncus effusus* in gypsum. For eleven weeks some of these young shoots were wholly encased in gypsum, at the end of which period they showed the peripheral zone of living cells thicker radially by two rows of cells than in normal shoots. In this case, cells that weeks before would have passed over into the dead, stellate form had, so far as cause can be discerned, been kept alive because they had not been subjected to the usual stretching from the normal growth of the stem [2].

The formation of the intercarinal canals in *Equisetum limosum* was delayed for a week or more by the application of a gypsum-cast to the base of a young shoot. The stem did not, outside the cast, increase subsequently in diameter; hence the cast prevented only longitudinal extension of the enclosed internodes. In *Zea Mais* the formation of the lysigenous canal in the vascular bundles was prevented in

[1] The method of applying these casts is described by Pfeffer, in Berichte d. K. Sächs. Gesellsch. d. Wissenschaften, December, 1892. The author, who learned the method from Pfeffer, has described it in Botanical Gazette, April, 1894, in an article entitled The Effect of Mechanical Resistance on the Development and Life-period of Cells.

[2] It is probable that another factor comes into play in this case; that is, that the cells live longer, not only because they are not stretched by adjacent tissue, but because they are prevented from making their own active and normal growth. This question has been discussed by the author in The Effect of Mechanical Resistance on the Growth of Plant-Tissues, Leipzig, 1893, and in The Effect of Mechanical Resistance on the Development and Life-period of Cells, Botanical Gazette, April and May, 1894.

like manner. The stem here did not increase in diameter after the cast was applied, but the enclosed internode, when examined, was but one-third the length of adjacent internodes. The small cells that are usually destroyed when the canal is formed were living and intact about the annular vessel.

The explanation of the prolongation of the life-period of the tissue in the foregoing five cases otherwise than as the result of relieving the tension, seems to me improbable. In the one case only of the leaves of *Allium Cepa* in gypsum-casts was there any appreciable constriction of the part in which the cells in question were preserved. When the constriction is considerable, it is conceivable that the cells might by regulatory means be kept alive for purposes of transport. But I have produced in several species of plants, by means of gypsum-casts, segments of stems with one-fourth the area in cross-section of the same stem above and below the cast, and all without an apparent effect on the transpiration or vitality of the plants. Such plants have been obliged, therefore, to carry their transpiration-current through a channel one-fourth as great as in other parts of the stem. It is thus demonstrated that, for ordinary transpiration, these plants do not need their full amount of xylem and conducting tissue. Hence it is pretty certain that the central cells in the segments of the Onion-leaves encased in gypsum were not kept alive for needs of transport, since the conducting channel in them was not greatly narrowed by the cast.

Finally, the cases of *Urtica dioica* and *Dahlia variabilis* may be again cited as furnishing evidence for the varying duration of the life of the pith according to the tearing action of the peripheral zones upon it. It will be remembered that in these two species the cavity appears in thick stems during primary growth, but in slender stems during secondary growth.

The life-period of cells cannot, however, be indefinitely prolonged by averting destruction due to tension: in fact, the destruction of cells by tension acting between tissues is an indication that such cells are near the end of their life-period. As already stated, etiolated leaves from the bulb of *Allium*

Cepa preserve all their cells alive for a considerable period after normal leaves become hollow. But it is also true that in such etiolated leaves the central parenchyma finally dies before the leaves die, and dies without collapsing. Again, the intercarinal canals in *Equisetum limosum*, though their formation has been delayed for a time by gypsum-casts which prevented the young internodes from elongating, finally appear within the limits of the casts as well as outside. In *Caltha palustris*, in whose stem a large lysigenous cavity appears during primary growth, the death of the pith is delayed by the use of gypsum-casts, but not altogether prevented, unless the cast is put about the stem when the latter is so young that the great prolongation of the life of the pith must, as will be shown later, be looked upon as a regulatory process. In the stems of this plant the tension is so much reduced by the use of the casts that the pith does not collapse when it dies, and we have the same condition as normally occurs in *Sambucus* and other plants.

In *Lamium garganicum*, *Myrrhis odorata*, *Archangelica sativa* and *Melianthus major*, precisely similar results have been obtained as with *Caltha palustris*. All of these plants normally form a lysigenous cavity in the pith during primary growth: all of them had casts applied about their stems before any pith-cells died, yet not so early as to prevent the pith-cells from attaining or nearly attaining their full size. In all such cases the life of the pith was prolonged from one to several weeks, but the pith then died *without collapsing*.

It is, of course, to be understood that to obtain these results, and to be able to make the statements given, very numerous experiments have been performed. Some of these experiments under somewhat different conditions have given results which are not stated in this place because they will best be considered later under another heading. The one fact which now demands special attention is the result that the pith died in all these plants but a short time later than normally and without being torn as it is usually; and this leads to the conclusion that such cells would not live much

longer than usually, were the normal tearing averted. In other words, the tearing shortens only by a few days or weeks what would otherwise be the life-period of the cells.

5. Effect of Prevention of the Extension of Surrounding Tissues.

As shown on page 406, it is possible for a cavity to begin schizogenously in secondary growth by the tension called forth in the contraction of cells or tissues due to loss of turgor while the surrounding tissues maintain their fixed position and size. It is also conceivable that if, in stems in which such a relation exists, the full primary extension of the vascular zone and the cortex be prevented by the early application of a gypsum-cast, there will arise less tension between pith and vascular zone when the former loses its turgidity during secondary growth. If the tension be thus averted it is quite possible that the pith-cells would live longer, since they would not be torn apart. And I am quite certain that many of my plants have demonstrated that this actually occurs.

Triticum repens, *Althaea taurinensis*, and *Eryngium planum*, which form a central cavity shortly after the cessation of primary growth, have had the cavity-formation deferred, but only for a short time, by laying around the stem an envelope of gypsum a few days before primary growth ceased. In *Triticum repens* the cast was applied to the rhizome. The lower internodes of *Vicia Faba* and *Dahlia variabilis*, in which the cavity appears subsequently to the beginning of secondary growth, have with similar treatment given essentially the same results.

But in many plants about whose stems gypsum-casts have been placed, the cavity-formation has been deferred for weeks and even months, with attendant phenomena which render it impossible that it should be the prevention of tension which has preserved the pith.

The first group of plants to be considered includes those which form a lysigenous cavity in the pith during primary

growth, and about whose stems casts were placed before any cavity existed. In *Urtica dioica* the pith has thus been preserved for three weeks within the cast after it had died outside the cast; the possible period of its preservation was not determined. *Althaea taurinensis* preserved its pith within the cast five weeks after its death outside the cast; *Lamium garganicum*, five weeks longer within the cast; *Dahlia variabilis*, thirteen weeks longer within the cast; *Vicia Faba*, sixteen weeks longer within the cast. In only one of these plants was the experimentation followed far enough to determine the limit of this extension of the vitality of the pith, and this is purely individual, so that we cannot base any general conclusion upon it. This individual was *Dahlia variabilis*, about whose stem a cast remained for six months. On examination there was found a very narrow strand of dead cells in the middle of the pith-cylinder. This period of six months, however, is as long as the ordinary life-period of this species; and this fact, coupled with the case of *Vicia* which preserved its pith sixteen weeks and was then bearing fruit, makes it almost certain that with proper conditions the life of a normally early-dying pith may be made coextensive with that of the individual.

With such an unusually long preservation of the pith there always goes a good growth of the stem, so that when the cast is removed its position is marked by a narrow neck connecting the much wider adjacent and normal parts. The same species that have been named as preserving their pith during such long periods have furnished examples of dying pith in shorter periods in individuals that made but poor growth after the application of the casts.

In these cases of remarkably long-preserved pith there is apparently another factor, besides the prevention of destructive tension, that is effective in giving the result, viz. the factor of regulation, in which the pith is called into use in the economy of the plant, probably to assist in transport through the narrow isthmus of the stem.

Such a regulatory action would be similar to that recorded

by De Vries[1] in a flower-stalk of *Pelargonium zonale*. Among the flower-buds on this stalk appeared a vegetative bud. The flower-buds were removed and the vegetative bud allowed to develop, resulting in the conversion of the usually short-lived flower-stalk into a permanent vegetative axis.

If any doubt remains as to the correctness of the view that the prevention of the destructive tension is not alone sufficient to account for the greatly prolonged vitality of the pith, that doubt can be removed by the following results of experiments:—In *Helianthus tuberosus*, *Sambucus nigra*, *Forsythia viridissima*, *Pterocarya fraxinifolia*, and *Juglans nigra*, the pith dies, and in the three last collapses also, from several to many weeks after secondary growth begins. In all of these plants there is a firm zone of xylem of secondary formation enveloping the pith before there is any indication of dying cells. Casts were placed about the stems of several individuals of each of these species after primary growth had ended. The cast could thus have no effect in lessening the tension between pith and more peripheral tissues, unless in secondary growth there were a displacement of the vascular ring toward the centre. But such displacement did not occur; the zone of mechanical tissue formed before the application of the casts was sufficiently strong to prevent it. In this series of experiments the stems that grew well—those that increased their diameter greatly in excess of the portion held within the cast—preserved their pith alive within the cast for a long period, while similar stems that made but small growth subsequently to the application of the casts preserved the vitality of the pith for a shorter period. Whether in these cases of flourishing stems the pith would live on indefinitely, as seems to be indicated for *Dahlia* and *Vicia Faba* already cited, was not determined in the experiments. But several individuals of *Helianthus tuberosus* showed a living pith within the cast at least six weeks after it had died outside the limits of the cast. Similarly, *Sambucus nigra*

[1] De Vries, Ueber abnormale Entstehung secundarer Gewebe. Jahrb. f. wiss. Bot. Bd. 22 (1891).

showed within the casts a living pith nine weeks after it had died outside the casts; *Forsythia viridissima*, *Pterocarya fraxinifolia*, and *Juglans nigra*, a pith living thirteen weeks longer within than outside the casts.

If the objection be raised here that the pith lives because of the general vitality of the stem, the query might be put as to why it should thus live within the limits of the cast but die outside. Moreover there is still another series of experiments that give positive evidence on this point, and they will now be discussed.

It will be remembered that *Dahlia*, *Vicia Faba*, and *Melianthus major* form a large cavity in their pith during primary growth. The last-named plant, when a cast is put about its stem before the cavity appears, remains solid over into secondary growth, but soon thereafter begins the formation of periderm in the inner part of the cortex, and thus by the death of the external cells removes the resistance of the cast and forms secondary tissue rapidly. At the same time the pith dies within the cast. It has lived longer than it would normally, but though the tension upon it is not increased, it dies, probably because the plant does not need it longer to aid in transport through the constriction. What *Melianthus* does for itself has been artificially reproduced in the first two of the plants named above. Both of these had casts placed about their stems before a cavity was present, and the casts were allowed to remain for several weeks. During this time there is formed, in the outer part of the pith of these plants, a zone of thick-walled cells which become mechanical and form thus a bar to either the subsequent expansion or contraction of the central mass of thin-walled cells. The casts were now carefully removed without injury to the stem. The former location of the cast was marked by a deep constriction, and the pith within this isthmus was living and much less expanded than above and below, where it was dead. If secondary growth had not already begun, it now started vigorously, so that in a very few weeks the constriction was obliterated. The pith meanwhile does

not enlarge; it is confined by its thick-walled, outer zone, and by the xylem-ring which forms immediately on the removal of the cast. If the plant is sectioned a few weeks after the removal of the cast, the pith is found dead within the former limits of the cast as well as in other places. There is no question but that the removal of the cast induced the death of the pith, since numerous experiments have shown that when the cast is left longer, the pith lives longer.

To my mind the only explanation of the facts recorded on the last few pages is this: The life of the pith is greatly prolonged by a regulatory process of the plant, the pith probably being used for transport through the narrow channel enclosed by the cast. If however the cast be removed so that the conducting tissue can be increased, the pith within the constriction dies, though it is neither stretched nor torn by the more peripheral zones.

SUMMARY.

The results obtained from the foregoing record of experiments and argument may be comprised in the following statements regarding the formation of lysigenous cavities:—

1. *Whether the cavity shall appear during or subsequently to primary growth depends upon the cessation or retardation of extension in the tissue where the cavity appears, relatively to the extension in the more peripheral tissues.*

2. *The initial cavity-formation in primary growth is always schizogenous, and may be schizogenous in secondary growth by the contraction of cells through their loss of turgor.*

3. *In cavity-formation during primary growth there are always two factors, a schizogenous and a lysigenous. When the tissue in which the cavity is to be formed ceases to expand early in primary growth, the schizogenous factor is large and the lysigenous small. When the tissue in which the cavity is to be formed does not cease to expand till late in life, the schizogenous factor is small and the lysigenous large.*

4. *In cavity-formation during secondary growth the*

schizogenous factor, if present, is always small, the lysigenous factor always large.

It need probably not be stated that there are many plants which begin the formation of cavity in primary growth and continue it over into secondary growth. This relation passes insensibly into that given in the next statement.

5. *There are species of plants in which some individuals form cavities in the pith during primary growth, others during secondary growth, the one condition or the other obtaining according to the cessation of growth in the pith during or subsequently to primary extension in the more peripheral zones of tissue.*

6. *The formation of a lysigenous cavity during primary growth may be somewhat deferred by preventing the normal primary extension of peripheral tissues.*

As a corollary, it may be added that the tension of tissues is a factor in limiting the life-period of cells which normally collapse in cavity-formation.

The evidence for this conclusion is, that in the leaves of *Allium Cepa*, kept from inflating by etiolation or by a gypsum-cast, the central cells live longer than normally; that the inner cells of *Juncus effusus* live longer when the shoots cannot expand; that the intercarinal canals of *Equisetum limosum* are deferred in formation when the elongation of the internode is prevented; that the canal in the bundles of *Zea Mais* is deferred in formation when the internode is not allowed to elongate; that several dicotyledonous plants preserve the vitality of their pith longer than normally when gypsum-casts are put around the stems so as to lessen the outward pull on the pith, but not to cause a great constriction during the period of primary growth; that no experiments or observations have controverted this conclusion.

7. *The formation of cavity during primary and secondary growth may be greatly deferred by preventing the extension of surrounding tissue. But in this case the result is to be referred to a regulatory process, probably to the use of the tissue concerned for purposes of transport.*

The evidence for this conclusion is, that in all those cases mentioned in the last paragraph of statement No. 6, the vitality of the cells in question was never prolonged more than a week or two except in the case of *Juncus*; that in plants in which the pith normally collapses it died without being torn when the tearing was prevented by surrounding the stems with casts; that the pith, which does not die till secondary growth has begun, was preserved alive when the casts were not applied till primary growth had ceased, the casts in this case not affecting the tension between pith and more peripheral zones; that the pith was never preserved long unless the stems encased made good growth; and, finally, that in stems grown for a period within casts the pith died without collapsing on removal of the casts, though the tension on the pith was thereby not increased.

The Traumatropic Curvature of Roots.

BY

VOLNEY M. SPALDING,

Professor of Botany in the University of Michigan.

With Plate XXII.

INTRODUCTORY.

THE name Traumatropism has recently been given [1] to certain phenomena observed to follow the infliction of wounds upon the tip of growing roots, which apparently are to be classed with geotropism, hydrotropism, and other corresponding reactions to external stimuli. Inasmuch as the subject has hitherto received comparatively little attention, and the existing literature, though limited, is contradictory, it has seemed desirable to investigate it experimentally. Such a study, provided definite conclusions were reached, might be expected to throw light on the question as to whether the observed phenomena are to be classed as mechanical movements or as movements of irritability, a question upon which hitherto there has been total lack of agreement. In the course of the work certain other and unlooked-for results, of theoretical interest, have been brought out and will be spoken of in their proper place.

The writer wishes to express his sincere thanks to Professor

[1] Pfeffer, Druck- und Arbeitsleistung durch wachsende Pflanzen, p. 374, Bd. xx. d. Abhl. d. math.-phys. Cl. d. Königl. Sächs. Gesellschaft d. Wissenschaften. Leipzig, 1893.

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Pfeffer, who in every possible way has encouraged and aided the investigation, both by personal attention and by means of the admirable facilities of the Botanical Institute of the University of Leipzig.

General Account of Phenomena.

If a radicle of *Lupinus albus*, or other suitable species, preferably when it has attained the length of two or three centimetres, is branded just back of the apex with some convenient instrument, such as a hot glass rod, the tip begins, in the course of an hour or more, to bend away, so that in a few hours the lower part of the radicle is strongly convex on the injured side. With subsequent growth the curvature continues, and within a day or two, in extreme cases, the tip may have described an entire circle, more or less. The same change of direction follows other modes of inflicting injury at the same point, such as cutting, cauterizing with nitrate of silver, and the use of still other destructive agents.

The whole course of events gives the impression of a sensitive organ that has received an injury and is avoiding further danger from the same source by bending away. Provisionally the reaction just described will be referred to as traumatropic curvature to distinguish it from a different curvature that is plainly mechanical. The latter appears just at the place where the injury is inflicted, and is a short, almost sharp, bend, with the concavity on the injured side. This mechanical bend may be produced by injury to the growing part of the root anywhere from within a millimetre to several millimetres from the extremity; the traumatropic curvature, on the other hand, follows only when the injury is close to the apex, within 1·5 mm., or sometimes a little farther back, as in the case of larger roots. A common case is represented in Fig. 1, in which the long traumatropic curvature, *a*, with the convexity on the injured side, is strongly contrasted with the short mechanical bend, *x*, which is always concave on that side.

The root thus operated upon continues its growth, if the conditions are favourable, but the injured part shows more

and more distinctly as a dead mass of tissue, which eventually is sloughed off, as shown at *x*, Fig. 17. The tip of the radicle becomes regenerated in the course of a few days, and presents a nearly or quite normal appearance, though more or less pronounced traces of the wound, in the shape of a scar, curvature, or other deformity, may persist for an indefinite time.

For the sake of clearness and brevity, reference has thus far been made only to the phenomena of traumatropism as seen in the radicles of seedlings; their manifestation in secondary and aerial roots will be considered in subsequent pages. The literature of the subject is reviewed in connexion with the discussion of experimental work.

Material and Methods.

The experiments reported in the following pages were continued through seven months, during which time hundreds of specimens were observed under different external conditions, which were carefully noted. Every effort was made to exclude sources of error by the use of control plants, the rejection of imperfect or diseased specimens, and the repetition of observations.

In the course of the work many different species were experimented upon, but the most prompt and satisfactory results were obtained with the primary root (radicle) of seedlings of Angiosperms, among them *Lupinus albus*, *Vicia Faba*, *Pisum sativum*, *Ricinus communis*, and *Zea Mais*, and the aerial roots of *Vitis gongylodes* and various species of *Anthurium*.

The different ways of wounding already referred to, and some others, have been followed. The most convenient method consists in the use of a small glass rod, heated to redness in the flame of a Bunsen-burner, and then brought in contact for an instant with the part of the root that it is desired to cauterize. Metallic copper and nitrate of silver serve the same purpose, but it is more difficult to limit exactly their action, and the results are, to a corresponding

degree, less definite. Removal of some portion of the tissue by cutting, notwithstanding the practical difficulty of cutting exactly to a given depth and in a certain direction, has been satisfactorily employed. Alcohol was used for the purpose of ascertaining what might probably be attributed to its use in Darwin's shellac mixture, but although its influence was strikingly manifest, it was found difficult to restrict its action to a known area and depth. Besides branding, the application of heat by bringing the tip of the root into contact with the side of a glass retort containing boiling water, and holding it there a certain number of seconds, gave interesting and definite results.

Roots that had been operated upon were commonly allowed to grow in water of the temperature of the room in which the work was carried on. This was kept during the day at approximately 18° C., but of course varied considerably in the course of twenty-four hours. Other roots were grown in moist air, but in general the results were less prompt and certain than when they were grown in water. In both cases they were suspended so that the radicle pointed vertically downwards. In still other cases, in order to retain as perfectly as possible the normal conditions, they were removed from the sawdust in which they had grown only long enough to inflict the wound, and were then returned to it.

In an extended set of experiments growth was suspended for a number of days, after previous wounding, by placing the radicles in plaster-casts in the manner described by Pfeffer [1]. Growth was also suspended by keeping the radicles in water at a temperature of 0° C. for some days, but the use of plaster-casts was found preferable. These latter experiments were varied by branding after removal from the casts.

The klinostat was employed as far as was necessary to demonstrate the independent, though contemporaneous, action of traumatropism and geotropism.

[1] Druck- und Arbeitsleistung, p. 6, et seq.

Experiments and Observations.

The record of experimental work is given without much attempt at classification, but the experiments are grouped and numbered in such a way as to admit of convenient comparison and discussion. Only a comparatively small number of the experiments actually conducted are here described, those being selected that give the clearest impression of the results as a whole. Brief comments are given in some cases where it has seemed better to call attention to important points in direct connexion with the experiments, than to postpone this to the pages devoted to formal discussion of results.

Miscellaneous Experiments with Radicles of Seedlings.

1. Twelve peas with radicles ranging from 2 to 4 cm. in length were branded with a hot glass rod as follows:—

Nos. 1–4 branded 1 mm. from apex of radicle.
„ 5–8 „ 2 „ „
„ 9–12 „ 3 „ „

When examined twenty-two hours later, it was found that Nos. 1 to 4 were all curved, with the convexity on the branded side, while all the rest, Nos. 5 to 12, with the single exception of No. 9, were sharply bent at the branded spot and were concave on that side.

2. Eight specimens of *Vicia Faba* with radicles ranging from 5·6 to 7·4 cm. in length were treated as shown in the following table:—

No. 1 branded 1 mm. from apex of radicle.
„ 2 „ 1 „ „
„ 3 „ 1·5 „ „
„ 4 „ 1·5 „ „
„ 5 „ 2 „ „
„ 6 „ 2·5 „ „
„ 7 „ 3 „ „
„ 8 „ 4 „ „

Special care was taken not to burn too deeply, but in every

case a small brown spot, where the tissue had been killed, could be seen. The radicles were allowed to grow in a damp atmosphere, and when examined twenty-four hours later it was found that in the case of those specimens branded not more than 1·5 mm. from the apex there was more or less traumatropic curvature, as shown in Fig. 2. Those branded more than 1·5 mm. from the apex, Figs. 3, 4, 5 (branded respectively 2, 2·5, and 4 mm. from the apex), showed no traumatropic curvature whatever. At the point of branding, in each case, the short mechanical bend was plainly seen. This is of course more conspicuous when it is far enough back to have considerable elongation of the root take place beyond it (Fig. 5), but the bend is essentially the same at whatever point it occurs, and whether or not traumatropic curvature takes place at the same time. (Compare Fig. 2, in which there is plain traumatropic curvature, with Figs. 3, 4, and 5, in which only the mechanical bend is seen.)

Similar results have followed so uniformly and in such a number of experiments with radicles of different species, that the following general statement may be made without qualification:—Traumatropic curvature follows when the radicle is branded close to the apex, but when the branding is done back of a certain point, which varies somewhat in different species and in *Vicia Faba* is very near 1·5 mm. from the apex, no such curvature results, and only mechanical bending takes place. This mechanical bending, towards the side branded, is seen in all cases, whatever the distance of the brand from the apex, provided it does not lie back of the zone of growth.

Incidentally attention should be directed to an effect of the disturbance of normal growth that is occasioned by branding, and is manifested by the swelling of that part of the radicle lying back of the zone of most rapid growth. This is shown most clearly in Figs. 2 and 4.

3. Similar experiments, in which nitrate of silver was employed instead of branding, show precisely the same results, with the same distinction between traumatropic and

mechanical curvature. Its action, however, is less certain than that of branding. Thus, in a not particularly favourable case, of seven radicles of *Vicia Faba* cauterized 1, 2, 3, and 4 mm. from the apex, three showed the sharp mechanical bend; the others gave no definite result.

4. Four specimens of *Lupinus albus* had a fragment of metallic copper placed on the slanting side of the apex of the radicle and were kept in damp air. Eight hours later all were deflected, showing marked traumatropic curvature, which was still more pronounced at the end of twenty-four hours.

5. To four radicles of *Lupinus albus* were attached, close to the apex, a very light and small fragment of filter-paper, about 2 mm. square, which was saturated with 96 p. c. alcohol. Seventeen hours later, having been left during that time in a damp atmosphere, three of the four were strongly deflected.

With two other specimens the experiment was varied by using a piece of blotting-paper, about 2·5 mm. square, saturated with 96 p. c. alcohol. Seven hours later both were strongly deflected.

6. In connexion with the foregoing, an attempt was made to ascertain whether the deflection was induced by the action of the alcohol or by the pressure of the paper. That it was the former appears from various experiments, among them the following:—A thin fragment of glass was placed laterally just back of the apex of the radicle of six specimens of *Vicia Faba*. In all six cases the glass continued to adhere for twenty-two hours, but none of the specimens showed evidence of traumatropic curvature. Thin pieces of mica were employed with thirteen specimens of *Vicia Faba* with the same result. Other light objects were used in the same way without deflection following.

7. The radicles of ten healthy specimens of sprouting peas, the radicles being about 5 cm. long, were held so that the tips would touch laterally the neck of a glass flask from which steam was issuing. The water had been heated for an hour or more. The temperature of the glass at the point where

the radicles were held was not accurately determined, but could not have varied much from 95° C. The tips of the radicles were held obliquely against the hot glass for five seconds, and then were allowed to grow in water of the temperature of the room. Seventeen hours later seven of the ten were curved away from the side to which heat had been applied. Of the three remaining ones, one was slightly bent away, or was doubtful, and two were bent in another plane.

Aerial Roots.

8. Ten of the aerial roots of *Anthurium* sp. growing in the moist air of the conservatory were branded in the usual way on the sloping side of the tip. Twenty-four hours later seven of the ten were strongly deflected, two at about a right angle, as shown in Fig. 6.

Microscopic examination shows that the root-cap constitutes a comparatively small proportion of the tip of the root, and does not extend far enough back to cover the curved part. It is continuous with the epidermis, into which it insensibly passes, there being no plain line of demarcation. In another specimen the curved part of the root extends to a still greater distance, so that the mechanical action of the root-cap is in the present case entirely inadequate to explain the curvature.

In *Anthurium*, differentiation of the tissues takes place at a very short distance from the extremity of the root. Tracheids were distinctly traced as far as *t*, Fig. 6. The curvature in the present case, therefore, takes place in tissues already differentiated, but making a rather rapid growth. This last is indicated by measurements made in connexion with the following experiment:—

9. Ten aerial roots belonging to the three species *Anthurium achranthum*, *A. hybridum*, and *A. Veitchi Andreanum*, were branded. Twenty-four hours later seven of the ten were traumatropically curved, four of them very strongly. The three that remained straight were found not to have grown appreciably. Those that had become curved

had grown in twenty-four hours quite rapidly, the increase in length ranging from 2 to 8 mm.

10. Eleven aerial roots of *Vitis gongylodes* were branded in the usual way at 10 a.m. At 3.30 p.m. all showed plain deflection, one being curved at nearly a right angle. The next day the traumatropic curvature was more pronounced, as was also the short mechanical bend at the point of branding.

A longitudinal section shows that the root-cap in this species is inconspicuous, its entire length from the extremity to where it is continuous with the epidermis being 0·5 mm. or less. As in the case of *Anthurium*, the traumatropic curvature lies so far back of the extremely small root-cap that the mechanical action of the latter is not to be thought of as its cause.

11. Two roots of *Anthurium* sp. were wounded as a result of their growing against the sharp point of a pin previously fixed obliquely in their path. Five hours after the pins had been placed in position the roots were examined and both were beginning to turn away. One had already been penetrated by the pin to a depth of approximately 2 mm. After twenty-three hours this one was still more strongly deflected, the other not having continued to curve much farther from its path. Deflection was also observed to take place as the result of merely pricking the growing-point with a clean needle, which was immediately withdrawn after making the wound.

Extent and Direction of Wound.

While the experimental work was in progress it soon became evident that the nature, direction, and extent of the wound constitute an important factor that had received too little attention. Accordingly an attempt was made to observe and compare with some degree of precision the results of wounding by cutting in different ways.

If the tip of a root is cut off square across, it does not exhibit traumatropic curvature, but if cut obliquely it becomes

curved, provided the cut is made to the right depth, as appears from the following experiments :—

12. The radicles of six specimens of *Vicia Faba* were cut as indicated in Fig. 7, two along the line *a a*, removing a small portion of the growing-point, two along the line *a' a'*, so that a small portion of the root-cap alone was removed, and two along a line midway between these, approaching very closely the growing-point, but lying in the tissue of the root-cap. When next examined (time not stated in laboratory notes, but within twenty-four hours), the first two were found to be strongly deflected—'bent up like a hook'—while the remaining four were either straight or so slightly curved as not to suggest the effect of cutting.

13. The experiment was repeated with four specimens of the same species, the radicle of one being cut along the line *a a*, those of the three others to different depths along lines parallel to *a a*, but with care not to cut deeper than the root-cap. Seventeen hours later the one deeply cut was strongly curved away; the rest showed no change of direction. Similar experiments, which it is unnecessary to record at length, were repeated with like results.

It is plain that, in order to induce traumatropic curvature with certainty by oblique cutting away of tissue at the apex, the cut must be made deep enough to affect the growing-point itself. It is perfectly certain that the root-cap may be cut deeply without curvature following.

Location of Sensitive Tissue.

The preceding experiments lead to the inference that the tissue lying just beneath the root-cap is sensitive, and receives a stimulus to which, after induction, the root responds by bending. To test this farther, and to locate more definitely, if possible, the sensitive tissue, the following experiments were performed :—

14. Ten specimens of *Vicia Faba* with vigorous radicles from 1 to 2 cm. long were selected and their root-caps removed with a sharp razor. The operation is a delicate one,

but after some practice can readily be performed, at least to the extent of making sure that no more than a very insignificant part of the root-cap remains. After the removal of the root-cap the tips were branded in the usual way and the radicles allowed to grow in water. Twenty-three hours later seven of the ten radicles were strongly deflected. Of the remaining three, one was slightly deflected, one was straight, and one was slightly curved towards the branded side. The appearance of the seven was so striking that there could be no hesitation in pronouncing this a plain case of traumatropic curvature. Microscopical examination showed that all of the thicker part of the root-cap was removed, or was subsequently killed by branding. There is absolutely no reason to suppose that the extremely small amount of tissue remaining as root-cap is the active agent in bringing about curvature.

15. Twenty healthy specimens of *Vicia Faba* with radicles varying in length from 2 to 4·5 cm. were cut, or stabbed, with a sharp-pointed lancet, without removing any of the tissue of the root-cap.

(*a*) Ten were cut close to the apex in a tangential direction, care being taken to make the cut as shallow as possible, so that it was certain that only tissues of the root-cap were wounded.

(*b*) Six were cut in the same way, but much deeper, so as to make sure of wounding the tissue of the growing-point, the instrument still being held tangentially.

(*c*) Four were also wounded deeply, but the instrument in this case was held radially, so as to disturb the tissue of the root-cap as little as possible, though it must nevertheless result in cutting a considerable amount of tissue both in the root-cap and in the growing-point.

An examination of these different lots, five hours later, showed that of the first lot of ten specimens, five were deflected; of the second lot of six specimens, five were deflected, and the sixth probably also, but doubtful; and of the third lot of four, three were deflected, the fourth being doubtful. Comparing the three lots and noting amount of curvature

and the number plainly deflected in each lot, there could be no doubt that those wounded deeply enough to cut the growing-point itself showed more pronounced traumatropic curvature, and in a greater number of cases, than those in which the root-cap alone was wounded.

Suspension of Growth and its Relation to Traumatropic Curvature.

Growth, as already shown, is a necessary condition of traumatropic curvature, and it is of interest to ascertain whether, if growth is suspended by artificial means, curvature takes place when it is again resumed. In view of the fact that differentiation of tissues continues in spite of the suspension of normal growth, it might well be questioned whether the conditions induced by the infliction of a wound still induce curvature, notwithstanding the histological changes that have meantime taken place. Accordingly, repeated experiments were made by first branding radicles and then confining them in plaster-casts for longer or shorter periods, after which they were released and allowed to resume their growth. The results were so striking that it seems worth while to give a considerable number of them in detail. The experiments were begun on January 8, 1894. Three species were employed, viz. *Lupinus albus*, *Vicia Faba*, and *Zea Mais*. Vigorous specimens were selected, when the radicle was making a strong and normal growth, usually when it was about 2 cm. long, though some longer and others shorter than this were used.

16. Three radicles of *Lupinus albus* were branded and placed in plaster-casts, after which they were left in moist sawdust twenty-two hours, care being taken, as in other cases, to have them stand vertically and thus avoid complications due to geotropism. At the end of the twenty-two hours they were released from the casts and allowed to grow in water. In one hour and ten minutes curvature could be detected, and at the end of twenty-four hours, when next examined, all three had become strongly curved. Fig. 8 shows

one of these that had made a vigorous growth, as it appeared thirty hours after release from the cast.

Three other specimens of the same species, in which the experiment was varied merely as regards intervals of time, gave the same results. The radicles were kept in casts seven hours, and fifteen hours after their release were strongly deflected, the tip of one being nearly horizontal.

17. Three specimens of *Lupinus albus*, branded as usual, were kept in plaster-casts forty-seven hours. One hour and twenty minutes after their release two were plainly beginning to curve. Fig. 9 represents one of these as it appeared six hours, and Fig. 10 forty-eight hours, after release from the cast. The third radicle remained straight, apparently as the result of burning too deeply. All three, when examined two days subsequent to their release, were seen to have become regenerated.

18. Two specimens of *Lupinus albus* that had been in casts seventy-two hours after previous branding were released and allowed to grow in water twenty-four hours. At the end of this time one showed distinct deflection, though the curve extended only a short distance from the extremity, as shown in Fig. 11. From the extreme point of the tip to the middle of the bend was 2·5 mm. The other specimen was doubtful, though possibly slightly curved.

19. A specimen of *Lupinus albus*, the radicle of which had been in a cast 146 hours after previous branding, was released, and twenty-two hours later was seen to be strongly deflected (cf. Fig. 12, in which x is the branded spot, still easily recognized). Two other specimens of the same lot were removed 150 hours after placing them in casts, and nineteen hours later were slightly curved.

20. A specimen of *Lupinus albus* that had been in a cast eight days and six hours after previous branding was released and examined after it had been allowed to grow in water eighteen hours. At the end of this time it had grown but little in length, but was plainly curving away (Fig. 13). The short mechanical bend at x appeared as usual. The

behaviour of the radicle was in general the same as in cases in which it was simply branded and placed in water, except that after the long period in the cast the traumatropic curve was very near the end. The root-cap was ruptured, shreds of it still remaining attached, and the fact that internal changes had continued, while increase in length had been stopped, was still farther indicated externally by the irregular surface and the compact structure of the tip of the root. Twenty-three hours later, i.e. forty-one hours after its removal from the cast, the radicle was examined and drawn (Fig. 14). Comparing this sketch with the preceding one, both drawn natural size, it is seen that the lower part of the radicle, in something less than twenty-four hours, had elongated 7 mm. The burnt tissue at x is now sharply delimited, regeneration already being far advanced.

21. Two specimens of *Zea Mais* were removed from casts in which the radicles had been confined seventy-two hours after branding. After growing in water twenty-four hours, both were deflected. The one that had grown most is represented in Fig. 15. The other measured only 4 mm. from the bend to the extremity of the tip, but it was quite as distinctly curved, and at about the same angle as the one figured. Both were straight when taken out of the casts, and were not plainly bent five hours later. Figs. 16 and 17 represent the root as it appeared forty-eight hours after removal from the cast. At the end of this period (cf. Fig. 17) regeneration was far advanced, although a piece of dead tissue remained that was afterwards thrown off. A shallow groove could be readily traced from a to x on the branded side, and after ninety-six hours this could still be followed, though becoming less and less distinct, as far as the tip.

22. Two specimens of *Vicia Faba* were branded and placed in casts, in which they were allowed to remain twenty-nine hours. When examined sixteen hours after release from the casts, having grown meantime in water, both were strongly deflected. As in the case of other species confined for a considerable time in casts, it was observed that the curva-

ture was nearer the extremity than when the radicles are allowed to grow freely after branding. This is regularly observed, and is due to the fact that while increase in length is mechanically prevented, differentiation of tissue goes on, so that the tissue capable of lengthening is pushed forward more and more, until it extends but a very short distance from the apex.

This series of experiments establishes the fact that the changes induced in the act of branding, that would ordinarily be followed by traumatropic curvature in the course of from one to a few hours, remain effective for periods ranging from seven hours to more than eight days, if growth is mechanically prevented during those periods.

23. Reversing the order of procedure by branding after removal from the cast is still followed by deflection, as is shown by the following:—

Three specimens of *Lupinus albus* that had been in plaster-casts for twenty-six hours were released and branded. When next examined, twenty-three hours later, all three showed strong traumatropic curvature, one, as shown in Fig. 18, being bent nearly at a right angle. After another twenty-four hours this specimen presented the appearance shown in Fig. 19, which indicates a considerable, though not particularly rapid, growth of the part beyond the curve.

24. Two specimens of *Lupinus albus* that had been in casts two days and eight hours were released and branded. When examined sixteen hours later both were strongly curved, one nearly at a right angle. Here, again, it was noticeable that, after the release of the radicles from the casts, the zone of growth was very short.

25. In connexion with the preceding experiments, and to serve in some measure as a check, it seemed desirable to ascertain what would follow artificial suspension of growth by other means, such as lowering of temperature.

Seven specimens of *Lupinus albus* were branded in the usual way and then placed in water at 0° C, in which they were allowed to remain forty-eight hours. When taken out

at the end of this time the radicles were straight. They were then placed in water at approximately 18° C. Twenty-four hours later three of the seven were strongly deflected, two were straight, and two doubtful. One of the doubtful ones afterwards became deflected. Exact measurements were not made, but it appeared that the specimens that failed to curve did not grow, probably having died while in the ice-cold water.

26. Two suppositions (if we exclude mechanical hypotheses) are possible in connexion with the foregoing experiments: first, that the injured tissue of the tip acts as a constant irritant; or second, that by induction an influence is promptly transmitted to the zone of rapid growth, where it remains effective long after the infliction of the wound, it may be for a period of some days. To obtain evidence on this point the following experiment was conducted:—

Seven specimens of *Lupinus albus* with healthy radicles about 1·5 cm. long were branded in the usual way and immediately placed in casts. At the end of twenty-four hours they were removed from the casts and decapitated, care being taken to remove just enough of the tip to be sure to take away the burnt tissue and as little more as possible. Directly after decapitation they were again placed in casts for twenty-four hours. At the end of this period they were removed from the casts, their length measured, and then they were allowed to grow in water at the temperature of the room. All were perfectly straight except No. 7, which was slightly bent away from the burnt side. The table gives their length when placed in water and also twenty-four hours later.

No.	Length.	Length after 24 hours.
1	14·5 mm.	26 mm.
2	17·5 „	25 „
3	13·5 „	16·5 „
4	15 „	30 „
5	15 „	32 „
6	16 „	18·5 „
7	15·5 „	20 „

When examined twenty-four hours after removal from the casts the following was found to be the condition :—

No. 1 distinctly deflected.
„ 2 strongly curved towards branded side.
„ 3 „ deflected.
„ 4 straight.
„ 5 „
„ 6 deflected.
„ 7 deflected obliquely.

Of the seven radicles, then, the two that had made the longest growth were straight, and of the five remaining ones *four* were deflected and *one* curved towards the injured side.

27. Eight specimens of *Lupinus albus* were branded and then placed in casts, in which they remained forty-three hours. They were then removed from the casts and divided into two lots of four each, the first lot being decapitated and the second not. When examined after growing in water twenty-four hours it was found that all were alive and had grown quite uniformly, the greatest increase in length being 9·5 mm. and the least 5 mm. Of the four decapitated specimens two were quite strongly curved, but obliquely instead of directly away from the branded side. Of the four control specimens that were not decapitated three were strongly deflected, the fourth remaining nearly straight and appearing on closer inspection not to have been burnt enough.

From the results of these two experiments (26 and 27) it is clear that decapitation immediately after removal from the casts of roots previously branded does not necessarily prevent traumatropic curvature. That it should be less regular might naturally be expected. Evidently the presence of the root-tip, with at least the greater part of the burnt tissue, is not necessary. So far as the evidence goes it favours the view that induction takes place when the growing-point is stimulated by wounding, and that the condition thus induced in the zone of rapid growth remains effective for a period of some days. As yet, however, it appears impracticable to

exclude absolutely the possible presence and continued action of some portion of the dead tissue without removing too much of the terminal portion of the root.

Discussion and Conclusion.

A review of the experimental work recorded in the preceding pages, together with a comparison with what has been done by others who have investigated the subject from widely different theoretical standpoints, indicates that we are now in possession of the most important facts, and that regarding these there is a fairly general agreement. It is in the interpretation of the facts that extremely diverse views are still held by leading physiologists. It remains, therefore, to examine the whole evidence, and to determine, if may be, the conclusion to be drawn from it. The work of other observers will first be reviewed.

Charles Darwin, with whom in this work Francis Darwin was associated, was the first to demonstrate the phenomena of traumatropism[1]. His ordinary method was to allow radicles of seedlings of different kinds to grow in moist air, and, while they were still very short, to fasten a bit of card laterally to the tip by means of shellac dissolved in alcohol. It was found that a large proportion of the radicles thus treated 'became bent, generally to a considerable extent, from the perpendicular and away from the side to which the object was attached.' This was the case, for example, with fifty-two out of fifty-five radicles of *Vicia Faba*. He interpreted this as a demonstration of the sensitiveness of the growing point to contact, but unfortunately did not pay sufficient attention to the action of the adhesive mixture employed, which later investigation proves to be an important factor. Various other experiments were conducted to prove in different ways the sensitiveness of the growing-point of the root. Thus 'thin slices were cut off parallel to one of the sloping sides of the apex, and out of the eighteen radicles

[1] Power of Movement in Plants. London, 1880.

thus treated thirteen were bent away from the cut surface after intervals of from twelve to twenty-five hours.' In still other cases nitrate of silver was used as an irritant, with similar results. He noticed the short mechanical bend in the opposite direction, at the place of injury, so that it is plain that he recognized two distinct forms of curvature, or bending, and attributed one to mechanical causes and the other to irritability[1].

Darwin's work is open to criticism in a number of particulars which have been pointed out by later writers, especially Detlefsen, Sachs, and Wiesner. His chief error, however, lay in the view that the extremely slight pressure of various light objects fastened to the slanting side of the tip is the real occasion of the curvature that follows. This unfounded conclusion constantly appears in the discussion of his experiments, and leads to assumptions which the experiments themselves were far from proving.

But notwithstanding these and possibly other errors incident to the first experimental investigation of the question, Darwin fully established the fact of the deflection of the root-tip, by processes already described, and gave a theoretical explanation of the fact. His interpretation included two distinct thoughts: first, that the apical portion of the root is sensitive; and secondly, that extremely slight pressure, or, as he expressed it, simple contact, is an irritant sufficient to induce deflection. The latter has been clearly disproved by my own experiments, as well as by earlier ones of Wiesner and others.

The 'Power of Movement in Plants' appeared in 1880. Of the various papers called forth by it, partly no doubt because of the striking form in which the conclusions were expressed, the first that calls for special notice is that of Detlefsen, 'Ueber die von Ch. Darwin behauptete Gehirnfunction der Wurzelspitze[2].'

Detlefsen employed the various means that Darwin had used to induce deflection of the radicle, and introduced some

[1] Power of Movement in Plants, p. 151.

[2] Arbeiten des Bot. Inst. in Würzburg, Bd. II, 1882.

others. Thus it was found that deflection followed when silver nitrate, shellac-solution, thin pieces of glass, copper sulphate, and caustic potash were applied laterally to the tip of the radicle, and that the same result followed when the radicle was wounded at this point by cutting or by the application of a hot glass rod. It is assumed by Detlefsen that in all these cases the treatment results in injury or death to the cells touched, whether by cutting off the necessary supply of oxygen, as when bits of glass are attached, or by direct killing through the agency of heat or a poison. The curving of the root as the result of such injury he holds to be strictly mechanical. 'The mode of curvature is always the same, whether the root-cap alone is wounded, or a larger or smaller part of the punctum vegetationis is destroyed. Therefore the cause of the curvature can only be injury to the root-cap.' The curvature is explained as due to changes of tension. The tissues of the root-cap are stretched over those lying beneath, and when the root-cap is cut through, or is otherwise injured, the tissues just beneath extend more rapidly than those on the opposite side, hence the resulting convexity of the side operated upon.

But as the root-cap extends a considerable distance from the apex in the roots he employed (often 5 mm. or more in *Vicia Faba*), injury above the tip should produce curvature in the same direction, while as a matter of fact the reverse is the case. The difficulty was perceived by Detlefsen, but not cleared up. He states that he did not succeed in producing a curvature by branding more than 1 to 1·5 mm. from the apex, but did accomplish this by making fine transverse incisions, although in the case of three oak-roots, the only ones reported specifically, 'the curvature was not very pronounced.' Yet it is upon such evidence that the case was decided by Detlefsen, and that Sachs, accepting the proof as final, based the criticisms of Darwin's work that have been given such wide publicity[1]. It is enough at this point to say that it has now been proven experimentally that traumatropic

[1] Vorlesungen über Pflanzenphysiologie, 1882, pp. 843, 879, 880.

curvature takes place when the root-cap has been removed, and that in other ways the erroneousness of Detlefsen's explanation has been fully shown.

Wiesner[1] reviewed and criticized Darwin's work shortly after its appearance, and some three years later published the results of further investigations of his own[2].

The experimental part of the latter work is devoted chiefly to a study of the behaviour of radicles grown in moist air and in water, and to the results of plasmolysis. As already stated, Wiesner showed that Darwin was mistaken in referring the curvature to simple contact, and his experiments in this direction are conclusive. From others, which it is not practicable here to review at length, Wiesner reasons that the removal of the root-tip causes a change (afterwards more specifically defined) in the cells lying above it, which results in their membranes becoming more extensible. If, now, an intact root is injured on one side of the tip, the cells lying on that side just above the injured tissue show this increased 'ductility' of the cell-membrane and corresponding rapid growth resulting in curvature. His conclusion, which applies also to geotropic curvature, is that 'the irritation-hypothesis set up by Darwin, according to which the growth-movements of the root proceed from the assumed irritable tip, is proven to be untenable.'

From a careful review of Wiesner's experiments I am quite unable to determine how they support the purely hypothetical explanation which he gives. The assumption is that when a root is wounded on one side of the apex, the food-materials that would naturally go to the injured cells stop in those lying above them, and there effect a change in the cell-membranes by which they become more extensible. But this supposition can have weight only when the still active discussion of the mechanics of growth and curvature has resulted in more definite knowledge than we now possess:

[1] Das Bewegungsvermögen der Pflanzen. Wien, 1881.

[2] Untersuchungen über die Wachsthumsbewegungen der Wurzeln, Sitzb. d. K. Akad. d. Wissensch. Wien, LXXXIX Bd., 1884.

and how, if proven, it is in the slightest degree inconsistent with the existence of both sensitiveness and induction does not appear. 'Through lateral injury of the root-tip, the growing part of the root lying above the wound *undergoes a change* which results in greater ductility of the cell-membranes of this part.' But changes in the membranes of growing cells are brought about through the agency of their protoplasm, and the protoplasm of these cells cannot fail to be affected by the treatment the root has received and to react in one form or another. Wiesner's argument, therefore, does not affect the question of the sensitiveness of the root-tip, whatever bearing it may have upon the mechanics of growth-curvatures.

The papers just reviewed contain all of importance that has been urged against the view held by Darwin regarding the sensitiveness of the growing point of the root. On the other hand, Pfeffer, after the most extended investigation that has yet been made of the mechanics of growing roots, states his conviction that the mechanical explanations thus far given are insufficient to account for the phenomena, and that they belong rather to movements of irritation (*Reizbewegungen*[1]). The question being thus reopened, the evidence derived from the present experimental study remains to be examined.

As stated in the introduction, and shown in experiments 1, 2, and others, two distinct changes of form regularly follow lateral injury of the root-tip, one of which also follows when the root is wounded farther back. The latter, which in this paper is spoken of as the mechanical bend, results from structural and mechanical changes, and is to be clearly distinguished from the other (traumatropic) curvature, which is a proper growth-curvature[2].

[1] Druck- und Arbeitsleistung, p. 374.

[2] As the present paper is limited to a consideration of the traumatropic curvature proper, no attempt is made to discuss further the mechanical bend, nor what Wiesner calls the *Nebenkrümmung*, which is referred by him to changes of turgor. Still other curvatures are seen when growing roots are continuously observed, and confusion will be avoided by confining the attention to the curvature which Darwin investigated. The term traumatropism proposed by Pfeffer should

It has been shown (experiments 12, 13, and others) that traumatropic curvature follows an injury that extends to the growing-point, but fails to take place when even extensive injury is inflicted in which the growing-point is not involved. Thus we have seen that the root-cap may be wounded to a considerable depth without apparent effect, and that a wound which just opposite the punctum vegetationis is followed by deflection, produces no such result a millimetre, or even less than a millimetre, higher. On exclusively mechanical principles these facts remain unexplained, but become intelligible upon the supposition that the tissue of the growing-point is sensitive, and that the application of a stimulus here is followed by induction, the effects of which are manifested in the zone of rapid growth.

The behaviour of aerial roots (experiments 8–11) presents further evidence in the same direction. In these, precisely as in the radicles of seedlings, traumatropic curvature promptly follows injury, of whatever kind, that reaches the growing-point. Thus the action is the same whether the tip is branded and many cells destroyed in the process, or the injury is produced by the penetration of a sharp instrument driven into the growing-point of the root by its own elongation, with a minimum destruction of adjacent tissue. The inadequacy of Detlefsen's explanation, in view of the fact that the aerial roots employed are practically destitute of a root-cap, has already been pointed out. It is equally clear that the relatively very slight destruction of tissue caused by the introduction of the point of a needle leaves very little room for Wiesner's hypothesis.

Experiment 14, in which it was shown that the removal of the root-cap is still followed by traumatropic curvature after branding, has also an important bearing. The disturbance of the natural equilibrium must of necessity be so great, no matter how skilfully the root-cap is removed, that one might well doubt whether definite results could be obtained in

stand, since Wiesner's term 'Darwin's curvature' is extended by him to include what Darwin, so far as appears from his works, never observed.

this way. Yet out of ten roots thus treated, seven (probably eight) were deflected as usual. Again the evidence points to the sensitiveness of the punctum vegetationis, traumatropic curvature resulting in spite of most unfavourable mechanical conditions.

In some respects the most striking results are those observed to follow artificial suspension of growth after wounding (experiments 16–27). Through these experiments it was found that roots that have been wounded may be held in casts for a period of several days, and that traumatropic curvature still takes place when they are released and growth is resumed. Elongation of the root is prevented while it is confined in the cast, but the formation of permanent tissue continues, and the zone of growth is gradually pushed forward so that at the conclusion of the period of confinement it is much nearer the extremity of the root. As a result of this, the curvature, which is still in the zone of rapid growth, is very near the apex. In other respects, however, it takes place essentially as in roots that have not been confined. Whatever changes, then, as the result of irritation, have taken place in the tissues above the injured cells, they remain effective until setting the root free renders curvature possible.

Extraordinary as the case appears, however, it is only what might be expected if we regard the root as a living organism, and not simply as an aggregation of mechanical elements. The root is forcibly prevented from making a normal growth for a certain period, after which it is released and growth is resumed. Before confinement certain changes were artificially induced, in response to which curvature would have taken place had the root been free to grow. When, after confinement, growth again takes place the curvature follows. The whole course of events merely presents another, and on the whole a remarkable, illustration of the fact that, in some form, every vitally active organ responds through the reaction of its sensitive protoplasm to external influences, even though in the meantime the introduction of other and different conditions may greatly modify the outward expression of the reaction.

That the living organ is at the same time a most delicately adjusted piece of mechanism, and that its response to whatever acts upon it from without is executed on the strictest mechanical principles, every one understands, but the two facts are distinct, notwithstanding their frequent confusion, a confusion not confined to older writers.

While, then, an absolute demonstration may at present be impossible, the experimental evidence, as a whole, points so uniformly in one direction as to fully justify the belief that the growing-point of the root is sensitive, that induction follows its irritation, and that traumatropic curvature is the result. Upon this interpretation not only are the results of these particular experiments consistent with each other, but they are in harmony with what is thus far known of the reactions of sensitive organs in general, and of the structural and physiological characteristics of the root in particular.

Of special interest as an illustration may be cited recent studies of the propagation of heliotropic stimulus. It has been shown by Rothert[1] that in the case of certain leaf-structures, notably the cotyledons of different Grasses, the tip is most sensitive, and that heliotropic stimulus is transmitted from the tip to the lower part of the cotyledon, where, after an interval of time, the visible response by bending is observed. He emphasizes the fact that growth and sensitiveness to irritation are two entirely independent things, though both are factors upon which the curvature of the organ depends, a fact which also appears in the traumatropic curvature of roots. Other cases of *Nachwirkung* exhibited by different plant organs are so familiar as to require no reference.

Passing to the phenomena of geotropism as seen in the root, and comparing those of traumatropism, there is abundant evidence that the principle is identical. Darwin held that in geotropism we have another case of transmitted effects. This view was rendered probable by experiments of Brunchorst[2]

[1] Ueber die Fortpflanzung des heliotropischen Reizes, Ber. d. bot. Gesellsch. 1892, p. 374.

[2] Ber. d. bot. Gesellsch. II, 1884. p. 78.

and Firtsch[1], and recent critical studies of Czapek[2] prove beyond question the sensitiveness of the root-tip to gravitation and the existence of induction resulting in geotropic curvature. Thus the evidence obtained from various independent studies of the root and other organs, indicates that traumatropism falls into a general class of phenomena which includes those of heliotropism, geotropism, and other growth-curvatures, and that the same principles are to be applied in its study.

The phenomena of regeneration, to which reference has been made in the record of experimental work, affords additional and important evidence bearing upon the question in hand. It has been seen that roots wounded at the tip, provided the injury does not extend beyond the punctum vegetationis, rapidly become regenerated, and in the course of some three days, more or less, the tissue that had been destroyed is replaced by new, the restoration finally, in many cases, being so complete as to leave little, if any, trace of the wound. Such a process is necessarily, and in the strictest sense, physiological. A new and different set of conditions has been introduced, and to these, as to a specific stimulus, the cells of the punctum vegetationis respond in a specific manner[3]. In this response the energies of adjacent cells must necessarily be taxed to meet the demands of a condition that did not exist before the injury. Thus it is certain that in regeneration the growing-point responds to the very injuries that traumatropic curvature follows and from which it results. The phenomena go hand in hand, and it seems impossible not to regard them as two different forms of *Nachwirkung* resulting from the same cause.

The biological significance of traumatropism is also most clearly seen when considered in connexion with regeneration. In case of injury to the growing-point of the root it is essential to the welfare of the plant that repair should take

[1] Ber. d. bot. Gesellsch. II, 1884, p. 248.

[2] Unpublished investigations conducted in the Botanisches Institut of the University of Leipzig: see Annals of Botany, this vol., p. 317.

[3] Pfeffer, Pflanzenphysiologie, II, p. 172 *et seq.*

place as promptly and economically as possible. This is accomplished with remarkable rapidity by the process of regeneration. Meantime it is also important that while the work of repair is going on the root should avoid further contact with the source of injury, whether a sharp stone, a poisonous substance, or some other agent. This is brought about by traumatropic curvature, by means of which the tip of the root is turned well away from the injurious influence through which it had suffered. The facility with which secondary roots are formed might seem to indicate this as a more ready method of repairing the injury, but in the case of aerial roots these are largely wanting, and in the ordinary subterranean root-system it is apparently both more economical and more direct to repair the injured tip than to form new roots. When, however, for any reason the usual process fails, a satisfactory substitute is found in the formation of secondary roots. In fact, in certain species, as for example *Vitis gongylodes*, the process of regeneration was less frequently observed than the growth of secondary roots.

Thus it appears that in the phenomena of traumatropism and regeneration we have merely another chapter in the history of the manifold forms of response to external influences exhibited by living organs—influences that attract or repel, that work swiftly or slowly, that, like the mild warmth of spring, gently awaken the normal activities of the plant, or, like the fierce cold of winter or a burning caustic, wither and destroy, but in all cases influences to which the living protoplasm shows its acute sensibility. Self-defence, the gaining of every possible advantage with the least expenditure of energy, and the preservation of whatever has been found most useful, is here as elsewhere the underlying principle; and whether we regard the long period of developmental history in the course of which the higher plants have finally become able, in the face of many obstacles, to wrest their nutriment by means of special organs from the soil, or in the light of recent studies[1] we lay emphasis on their capacity for immediate

[1] See, for example, Sachs, Ueber latente Reizbarkeiten, Flora, 77 Bd., 1893, p. 1.

adaptation to the environment, in either case it is strictly in accordance with all we know of the habits of plants that the root should now be in possession of the power of turning in self-protection from an enemy it could not overcome, repairing whatever injuries have been inflicted, and then resuming its normal growth and functions.

Two questions of much theoretical interest present themselves in connexion with the one chiefly studied and thus far under discussion. The first, in regard to the mechanism of the movement exhibited in growth-curvatures of the root and other organs, has engaged the attention of physiologists for many years, and as yet is not satisfactorily explained. The present study throws no direct light upon it, but suggests the necessity in its further study of taking into account the various changes that take place while *Nachwirkung* is delayed by mechanical means. The second, regarding what is known as the latent period, i. e. the period intervening between stimulus and visible after-effect, has received such attention as could be given it, and our knowledge is thereby increased to the extent of the data accumulated in the various experiments with roots confined in casts. That the latent period may by such artificial means be extended for more than a week is a fact of sufficient physiological importance to warrant the more extended investigation which it is hoped may hereafter be given to it.

EXPLANATION OF FIGURES IN PLATE XXII.

Illustrating Prof. Spalding's paper on Traumatropic Curvature of Roots.

Fig. 1. *Lupinus albus* in germination, the radicle showing traumatropic curvature at *a*, and the mechanical bend at *x*.

Fig. 2. Radicle of *Vicia Faba* branded 1 mm. from the apex.

Fig. 3. Radicle of *Vicia Faba* branded 2 mm. from the apex.

Fig. 4. Radicle of *Vicia Faba* branded 2·5 mm. from the apex.

Fig. 5. Radicle of *Vicia Faba* branded 4 mm. from the apex.

Fig. 6. Aerial root of *Anthurium* sp. in longitudinal section while undergoing traumatropic curvature. Magnified three diameters. Tracheids may be distinguished as far as *t*.

Fig. 7. Diagramatic longitudinal section of the end of radicle of *Vicia Faba*. The section *a a* lies partly within the growing-point; the section *a' a'* lies wholly within the root-cap.

Fig. 8. *Lupinus albus*. Radicle thirty hours after release from a cast in which it had been confined twenty-two hours after previous branding.

Fig. 9. *Lupinus albus*. Radicle six hours after release from a cast in which it had been confined forty-seven hours after previous branding.

Fig. 10. Same specimen forty-eight hours after release from cast.

Fig. 11. *Lupinus albus*. Radicle twenty-four hours after release from a cast in which it had been confined seventy-two hours after previous branding.

Fig. 12. *Lupinus albus*. Radicle twenty-two hours after release from a cast in which it had been confined 146 hours after previous branding.

Fig. 13. *Lupinus albus*. Radicle eighteen hours after release from a cast in which it had been confined eight days and six hours after previous branding.

Fig. 14. Same specimen forty-one hours after release from cast.

Fig. 15. *Zea Mais*. Radicle twenty-four hours after release from a cast in which it had been confined seventy-two hours after previous branding.

Fig. 16. Same specimen forty-eight hours after release from cast.

Fig. 17. Same specimen enlarged, showing dead tissue *x*, about to be thrown off in the process of regeneration of the root-tip.

Fig. 18. *Lupinus albus*. Radicle twenty-three hours after release from a cast in which it had been confined twenty-six hours, and afterwards branded.

Fig. 19. Same specimen forty-seven hours after release from cast.

On the Double Flower of Epidendrum vitellinum, Lindl.

BY

C. H. WRIGHT,

Assistant in the Herbarium, Royal Gardens, Kew.

With Plate XXIII.

THE perianth of the normal flower of *Epidendrum vitellinum*, Lindl. (Fig. 1) consists of three equal sepals, and two lateral petals, broader than the sepals, and a smaller labellum. Both sepals and petals are orange-red, while the column and labellum are yellow. In the double flower (Fig. 2) the sepals are equal in size, but the two lateral ones have each a prominent keel, which is all but absent in the posterior one. The two lateral petals, instead of being broader than the sepals, as in the normal condition, are narrower than they. The labellum agrees in size, shape, and colour with the lateral petals, but its keel is more highly developed. The central portion of the flower is occupied by more or less petaloid organs. Of these the one opposite the posterior sepal (Fig. 9) is divided about two-thirds of the way down into two unequal segments, more or less expanded above. The inner margin of the larger segment is yellowish. The smaller segment is very slightly bifid at the apex, and is rolled forward so as to lie in front of the larger one. The base of this organ, which bears a few white hairs, is much thicker than that of either of

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those mentioned below, and possibly the two segments represent members of two different whorls. This is rendered more probable by there being no other indication of the member of the inner whorl adjacent to the smaller segment. The two other members of the outer whorl are alike (Figs. 7–8). Each is divided almost to the base into two unequal segments, the inner margin of each of which is bright yellow, thin and incurved, resembling the indusium of *Adiantum*. The anterior member (Figs. 10–11) of the inner whorl is also bi-lobed, but to a smaller extent than in the previous cases; the inner margins are incurved as before, but the outer alone are scarlet. The smaller of the two lobes has, on its outer margin, about one-third of the way from the base, a small tooth with a bundle of short white hairs projecting from its axil. Another member (Figs. 12–13) of this whorl is club-shaped, and slightly divided at the apex; one margin bears a small tooth, and the opposite one a bunch of white hairs; the greater part of the inner face is viscid, and has a central groove; in the apical part is a small depression. This member bears a certain amount of resemblance to the normal column, the apical depression being the anther, and the viscid inner face the stigmatic surface. The third member of the inner whorl is probably, as already stated, represented by part of that opposite the posterior sepal.

A transverse section of the pedicel a short distance below the ovary (Fig. 3) shows six fibro-vascular bundles arranged in a circle. A similar section taken close to the base of the ovary (Fig. 4) shows that the alternate bundles have branched and formed an inner whorl of three. Half-way up the ovary each of the primary bundles has again branched and formed a bundle of three, that is, eighteen for the entire ovary (Fig. 14). The cells beneath the placentae are filled with bundles of raphides, but no traces of ovules were to be found. At the top of the ovary (Fig. 15) the vascular bundles diverge, and bend away from the axis preparatory to entering the segments of the perianth. Each sepal and petal has five bundles. Beyond this point the bundles become very weak,

consisting of but a few spiral vessels, and branch in an irregular manner. A longitudinal section of a young bud (Fig. 5) shows clearly the four whorls in which the members are arranged.

Conclusion. The flower has attempted to assume a regular form by the arrest of the irregular parts (regular peloria). This has been accomplished in the case of the sepals and petals. The stamens have become free from one another, and more or less petaloid. The styles are probably still connate with the inner whorl of stamens. No trace of pollen or ovules was to be found.

The flowers, from which this description has been drawn up, were received in July, 1891, from Mr. W. Swan, gardener to G. C. Raphael, Esq., of Castle Hill, Englefield Green.

EXPLANATION OF PLATE XXIII.

Illustrating Mr. Wright's paper on the double flower of *Epidendrum vitellinum*, Lindl.

Fig. 1. Normal flower, natural size.

Fig. 2. Two 'double' flowers, natural size.

Fig. 3. Transverse section of pedicel, showing the six vascular bundles arranged in a circle. ×9.

Fig. 4. Similar section, but taken nearer the ovary, showing the formation of a whorl of three inner vascular bundles. ×9.

Fig. 5. Longitudinal section of a flower bud, showing one of the vascular bundles branching to form the inner whorl, shown in Fig. 4. ×3.

Fig. 6. Diagram showing the relative position of the members of the various whorls: *s.* sepals; *p.* petals. The numbers correspond to those of the enlarged figures of the same member.

Figs. 7–9. Members of the outer whorl. ×5.

Figs. 10, 11. Front and back view of the anterior member of the inner whorl. ×5.

Figs. 12, 13. Back and side view of a lateral member of the inner whorl. ×5.

Fig. 14. Transverse section of the ovary, showing the arrangement of the vascular bundles. ×9.

Fig. 15. Similar section taken near the top of the ovary, showing how the vascular bundles diverge. ×9.

Two Irish Brown Algae: Pogotrichum and Litosiphon.

BY

T. JOHNSON,

Professor of Botany, Royal College of Science, Dublin.

With Plate XXIV.

DURING an algological visit to the west coast of Clare in September, 1891, I found growing on the Kilkee rocks at low water, plants of *Alaria esculenta*, Grev., covered with brown tufts of fertile filaments looking very much like *Litosiphon Laminariae*, Harv. Professor Reinke of Kiel, to whom I sent material, concluded it was not this species, though very near to it, but a second and new species of the genus *Pogotrichum* of which he was then describing the type, *P. filiforme*, received from Heligoland. The Kilkee plant was accordingly, after examination and comparison, named *Pogotrichum hibernicum*. Reinke's diagnosis of *Pogotrichum*[1] is as follows:—

'Unverzweigte, büschelförmig beisammenstehende, fadenförmige Thallome von radiär gebautem Querschnitt und intercalarem Wachsthum. Vegetations-Fäden aus mehreren oder,

[1] J. Reinke, Atlas deutscher M. Alg., II, 3–5, s. 61, Tf. 41, Fig. 13–25.

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doch seltener, aus nur einer Längsreihe von Zellen gebildet. Pluriloculäre Sporangien intercalar in den Thallus eingesprengt, nur aus einigen der äusseren oder auch aus sämmtlichen Zellen eines Querschnitts gebildet; bei einreihigen Individuen durch Theilung einzelner Gliederzellen in viele kleine Zellen entstanden.'

P. filiforme, Rke., grows on the thallus of *Laminaria saccharina* and produces tufts 1–3 cm. long, the individual filaments being ·015 to ·06 mm. thick and arising on a basal layer which is a single layer of cells, disc-like, and clinging to the surface of the *Laminaria*-thallus. The filaments are not provided with lateral hairs. The diagnosis I gave of *P. hibernicum*[1] is as follows:—'Unbranched, tufted, filamentous thalli, 1 cm. long, of radially constructed cross-section, and intercalary growth. Basal cells of filaments rhizogenous and penetrating *Alaria*-thallus to give new tufts by endophytic hyphae. Filaments hairy, each one assimilative and reproductive, solid or subsolid. Chromatophores granular, 4–20, parietal.

'*Sporangia* unilocular and plurilocular in same tuft but not in same filament, chiefly in upper part of filaments, which part may become entirely converted into reproductive cells. Sporangia formed intercalarily, from individual joint-cells of thallus in uniseriate filaments or from superficial cells, or from all the cells of multiseriate filaments. Fate of zoospores unknown.'

Thus while *P. filiforme* consists of tufts of delicate, naked filaments, provided with plurilocular sporangia only, and arising from an epiphytic basal disc, *P. hibernicum*, on the other hand, consists of tufts of thick coarse filaments, clothed throughout their length with hairs, and possessing unilocular or plurilocular sporangia. A basal disc is not present, the numerous filaments of a tuft are closely compacted together, rhizogenous and endophytic, the intracortical hyphae being stoloniferous.

[1] T. Johnson, Proc. Roy. Dublin Soc., n. s. I. 1, p. 10, Pl. 1.

In the year 1849 the genus *Litosiphon* was founded by Harvey[1], on the suggestion of D. Moore, to include two plants, *Asperococcus pusillus*, Carm. and *Bangia Laminariae*, Lyngb. *Litosiphon pusillus* is characterized by Harvey as having 'fronds tufted, thread-shaped, very long, equal in diameter throughout, reticulated, clothed with pellucid hairs; spores scattered,' while *L. Laminariae* is described as having 'fronds stellately tufted, short, cylindrical, blunt, slightly tapering at the base, smooth (or hairy toward the apex), transversely banded, the bands close together, spores scattered, or several in each transverse band.'

The two species of *Litosiphon* show a relationship to one another very similar to that of the two species of *Pogotrichum* to one another. *L. pusillus* grows epiphytically in tufts on *Scytosiphon lomentarius* and on *Chorda filum*, and, so far as is at present known, produces unilocular sporangia only. The affinities of *Litosiphon* and *Pogotrichum* are so close that it was quite natural to suppose *P. filiforme* might be the unknown and missing plurilocular state of *L. pusillus*. Reinke convinced himself that this view was untenable. In discussing the affinities of *Litosiphon* and *Pogotrichum*, Reinke states[2] that so close are they that, had he known of the existence of '*P. hibernicum*,' he would probably not have founded the genus *Pogotrichum*. As, however, the *plurilocular* state of *Litosiphon* was not known, he thought it best to keep the two genera distinct, pending the more complete knowledge. I was fortunately able to examine herbarium-material of *L. Laminariae*, in which I saw plurilocular sporangia, like those of *P. hibernicum* in all essentials. Having regard, however, to the difficulties of examination, and the nature of the material, I preferred to leave the whole question open until freshly gathered material could be examined. Hence

[1] W. H. Harvey, Phyc. Brit., Pls. 270 and 245.

[2] J. Reinke, op. cit. S. 63. Murray, in Science Progress (no. 5, 1894), in commenting on my paper and expressing the opinion that I ought to have suppressed the genus *Pogotrichum*, states, inadvertently, that I *extracted* the information to which reference is here made, from Reinke by correspondence.

in my summary I say, '*P. hibernicum* is very near to, if not identical with, *Litosiphon Laminariae*.' I have within the past two or three years had several opportunities of collecting living material of *Alaria* infested sometimes by *P. hibernicum*, sometimes by *Litosiphon Laminariae*. Examination of this material has given me surprising results, explaining my difficulties in the examination of the herbarium-material, and justifying my earlier attitude.

Sporangia.—The filaments in the tufts of *L. Laminariae*, which are scarcely distinguishable to the naked eye from those of *P. hibernicum*, are fertile, generally abundantly so, and each filament possesses both unilocular and plurilocular sporangia (Figs. 5, 6, 7), a condition of things which is, I believe, unparalleled[1]. The lower half of the filament is usually purely vegetative, and the upper reproductive (Fig. 8). The unilocular and plurilocular sporangia have no regular arrangement; they are frequently intermixed in a most indefinite manner, standing singly or in groups, side by side, or separated by more or fewer sterile cells. They are derived from intercalary and sub-terminal superficial single cells or cell-surfaces. In all essential features the individual unilocular and plurilocular sporangia of *Litosiphon Laminariae* are like those of *Pogotrichum hibernicum*. They differ in occurring side by side on the same filament, not on distinct filaments as in *P. hibernicum*.

It will be convenient to speak of the filaments of *L. Laminariae* as *anisosporangiate*[2] in contradistinction to those of *P. hibernicum* in which sporangia of one kind only are

[1] On comparing the superficial appearance of a fertile part of a filament of *L. Laminariae* with a sterile part, it is easy to see how, in earlier times, with less perfect microscopes, the individual compartments of a plurilocular sporangium were mistaken, in filaments with *obvious unilocular sporangia*, for the large chlorophyll-grains, and *L. Laminariae* has thus continued to the present day to be described as possessed of unilocular sporangia only. Harvey in all probability saw the plurilocular sporangia, for in describing *L. Laminariae* he states that 'the (peripheral) cells sometimes separate into four smaller cells which occupy the same space,' a condition represented in the illustrations. Lyngbye and J. G. Agardh both speak of the *granula* being *quaterna* or *subquaterna*.

[2] The term *heterosporangiate* has already a definite signification.

present in a filament, and to which the term *isosporangiate* may be applied. The anisosporangiate state is, of itself, sufficient to justify the separation of *P. hibernicum* from *L. Laminariae* as a species. It is, however, not merely of systematic value. It is of interest in connexion with the discussion of the modes of reproduction[1] in Phaeophyceae by unilocular and plurilocular sporangia, and is not without significance in reference to theories of reproduction in the lower plants generally. No doubt a full knowledge of the fate of the contents of the sporangia would be of great interest.

Thallus.—A difference of considerable importance in discussing the affinities of these plants exists in the structure of the thallus. In *P. hibernicum* and in *L. Laminariae* the filaments are multicellular, usually pluriseriate. But while in *P. hibernicum* the internal cells are not markedly dissimilar to the external ones, in *L. Laminariae* the central axial cells are much larger than the peripheral ones, and by their horizontal cross-walls and subverticillate hairs give to the filaments a zoned appearance (Figs. 7, 8).

Vegetative reproduction.—*L. Laminariae* differs from *L. pusillus*, as *P. hibernicum* does from *P. filiforme*, in being of endophytic habit. The earlier description[2] of this habit in *P. hibernicum* will apply equally well to *L. Laminariae*. The filaments of a tuft are unbranched and to this extent unconnected; they are, however, at their lower ends in close contact with one another and more or less fused into a compact body of a subparenchymatous nature. There are to be observed, growing out from the superficial cells at the base of the filaments, rhizoidal septate hyphae which come into contact with the surface of *Alaria*, and can no doubt, as in so many Phaeophyceae, give rise to new *Litosiphon*-plantlets. On making a vertical section of *Alaria* through the anchorage of a *Litosiphon*-tuft, the individual filaments and rhizoidal hyphae of *Litosiphon* are seen to penetrate into the *Alaria*-thallus, to creep and ramify between the cortical and the

[1] E. Bornet, Note aux quelques Ectocarpus.

[2] T. Johnson, Proc. Roy. Dublin Soc., n. s., I. 1, p. 2.

medullary cells (Fig. 9). These endophytic or intra-cortical hyphae can be traced from one surface to the other of *Alaria*, and there is every indication that they can, after creeping some distance, emerge at the surface of *Alaria* to form new tufts of *Litosiphon*. I took the opportunity, in describing similar phenomena in *P. hibernicum*, to refer to an important paper on parasitic Brown Algae by C. Sauvageau[1], and content myself now with saying it is an important paper which has not received the attention in this country which its importance deserves. I have no doubt of the distinctly injurious effects of the innumerable tufts of *Litosiphon* on the thallus of *Alaria*. This injury is not due, apparently, to any special absorption of food-matter by parasite from host, but rather to an extreme type of 'Raumparasitismus.' I have, for example, seen at Bundoran, this September, plants of *Alaria* in which the thallus, or what was left of it, was full of holes due to *Litosiphon*-tufts, and others in which very little was left but midrib, and ragged ribbons of infested thallus. Whether this form of parasitism produces any degradation or not in *Litosiphon*, I cannot say.

Summary.—The larger size of the filaments, their more distinct subarticulate appearance, and the large medullary cells of the filaments in *L. Laminariae*, together with the differences in the arrangement of the reproductive organs in *L. Laminariae* and *P. hibernicum*, are sufficient to justify their separation from another as species[2]. If the differences are not of generic rank and suppression must occur, I should prefer the execution to take place by the hand of Reinke, who, in founding *Pogotrichum*, pointed out the possibly modifying effects of a fuller knowledge of the nature of *Litosiphon*[3].

[1] C. Sauvageau, Sur quelques algues phéosporées parasites, in Journal de Bot. vi., 1892.

[2] Batters in Grevillea (104, p. 118) had already, in reviewing my former paper, expressed this opinion tentatively.

[3] Prof. Reinke tells me his eyesight is now such that he is quite unable to carry on finer microscopic investigations: even writing is painful. If *Pogotrichum* be suppressed, he for one will rejoice that there is one useless genus the less in the world.

P. filiforme.	*P. hibernicum.*	*L. pusillus.*	*L. Laminariae.*
1. Tufts of unbranched filaments in all.	Tufts of unbranched filaments in all.	Tufts of unbranched filaments in all.	Tufts of unbranched filaments in all.
2. Filaments naked.	clothed with hairs.	ditto.	ditto.
3. Epiphytic on *Laminaria saccharina.*	Endophytic in *Alaria esculenta.*	Epiphytic on *Scytosiphon* and on *Chorda.*	Endophytic in *Alaria esculenta.*
4. Filaments mostly monosiphonous or one-cell-seriate.	Mostly polysiphonous.	ditto.	ditto.
5. Filaments isosporangiate. Plurilocular sporangia only known.	Filaments isosporangiate. Plurilocular sporangia known.	ditto 1-locular sporangia only known.	Filaments anisosporangiate. 1-locular and plurilocular sporangia known.

THE LABORATORY, Glasnevin.

EXPLANATION OF FIGURES IN PLATE XXIV.

Illustrating Prof. Johnson's paper on two Irish Brown Algae.

Fig. 1. Tufts of *L. Laminariae* on the thallus of *Alaria*, nat. size.

Fig. 2. A single large tuft.

Fig. 3. Cross-section of *Alaria* through bases of several tufts of *L. Laminariae*. × slightly.

Fig. 4. Sterile cells of surface of sterile of filament. The large chromatophores are shown. × 320.

Fig. 5. Surface of fertile part of filament showing unilocular *u. s.*, and plurilocular sporangia *p. s.*, mature and dehisced. × 320.

Fig. 6. Longitudinal section of fertile part of the filament showing the sporangia and the large sterile medullary cells, *m*. × 320.

Fig. 7. Transverse section of filament showing same as Fig. 6, and sterile surface cells (*s. c.*); *b.* a younger filament. × 320.

Fig. 8. A single filament (plantlet), showing typical portions from base to apex: (1) is the base showing very few hairs and the detached connection; (2) shows the subarticulate appearance due to the large medullary cells and the hairs; (1) and (2) are sterile; (3) most of surface of fertile cells; (4) apex of filament, sporangia dehisced mostly, medullary cells bulging. × 70.

Fig. 9. Anchorage of *L. Laminariae* (after Reinke), showing bases of three plantlets, *L*, with rhizogenous filaments, *r*, and intra-cortical hyphae, *h*, in the thallus of *Alaria*, *A*. × 400.

Fig. 10. Transverse section of *L. pusillus* showing unilocular sporangia, which are the only ones known. × 320.

NOTES.

ON APOSPORY AND PRODUCTION OF GEMMAE IN TRICHOMANES KAULFUSSII, Hk. and Gr.—In the first volume of the Annals of Botany [1] I described how outgrowths may appear on the margins, and even on the surface, of the fronds of *Trichomanes alatum*, Swartz. These take the form either of ribbon-like bodies, or of filaments, and it was concluded that they were of the nature of prothalli produced by aposporous growth, since they had the characters of the Hymenophyllaceous prothallus, and even bore antheridia; the dark brown rhizoids were a marked feature on the filamentous growths. A further notable character was the production of numerous gemmae, similar in form to those previously described by Cramer [2], and by Goebel [3], on prothalli of Hymenophyllaceae.

On a very fine plant of *Tr. Kaulfussii*, Hk. and Gr., growing at Kew, I recently found outgrowths of a similar nature to those of *Tr. alatum*, produced in prodigious quantities; the leaves which bear them appear to be destitute of sporangia. I have only noted filamentous outgrowths in this species, none developing into flattened expansions, as so frequently occurs in *Tr. alatum*. It is to be noted in this connexion that the plant was grown in a closed case in a densely shaded position, and it has been recently shown by Klebs [4] that in other ferns cultivation in weak light induces filamentous development, and frequent adventitious branching; in fact, just such developments as were found on the Kew plant of *Tr. Kaulfussii*. The filamentous growths originate from single marginal or superficial cells of the frond, and bear lateral rhizoids of a dark brown

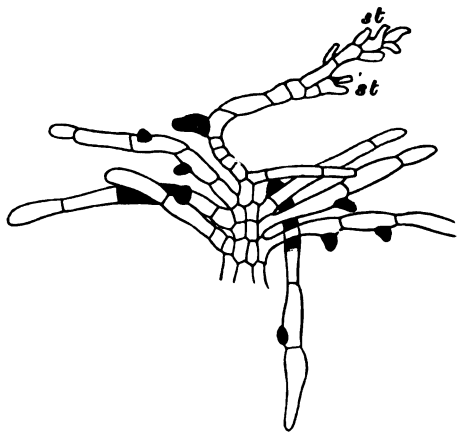
[1] Preliminary note, Annals of Botany, Vol. i, p. 183. Full description with Figures, l. c., p. 278.

[2] Denkschr. d. Schweitz. Nat. Ges. XXVIII, 1880.

[3] Ann. Jard. Bot. d. Buitenzorg. Vol. vii, p. 72.

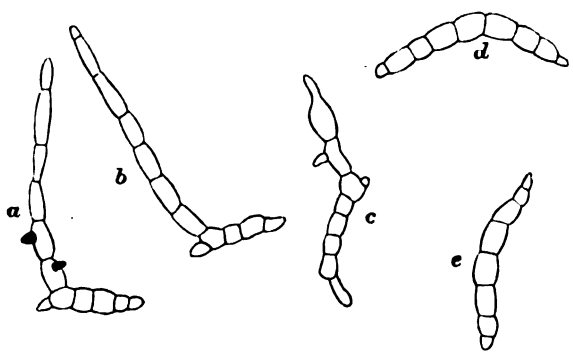
[4] Biologisches Centralblatt. Bd. XIII, p. 641, &c.

colour, similar to those on other prothalli of *Trichomanes*. As in the case of *Tr. alatum*, the prothalloid growths bear on their apices short branchlets, or sterigmata (Woodcut 3); these occur singly or in tufts,



WOODCUT 3.

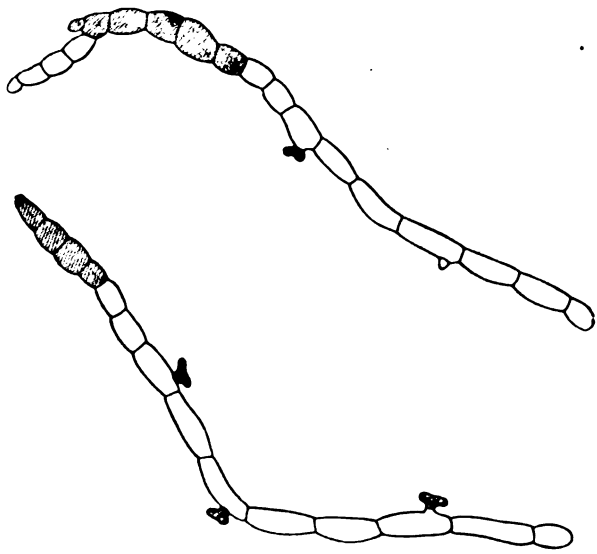
and balanced upon the apex of each in the most elegant manner is a single gemma, of spindle-form, similar to those in *Tr. alatum* (l. c. Plates XV, XVI). The attachment of these is very slight, and the gemmae very easily displaced.



WOODCUT 4.

That the gemmae germinate readily is seen from the fact that on the specimen kindly sent to me by the Director of the Royal Gardens,

Kew, certain of the gemmae already showed many-celled filamentous outgrowths, and the same was the case with the gemmae on another specimen sent by Dr. Winter from Brighton (Woodcut 4). The germinating filaments most frequently grow out laterally (*a*, *b*, *c*), but sometimes from the end of the spindle (*d*, *e*). But after the first steps the further growth is exceedingly slow:—Woodcut 5 shows the result of culture for about six months at a moderate temperature. The filaments show characters similar to those of *Tr. alatum*, and though no sexual organs have been found upon them in *Tr. Kaulfussii*,



WOODCUT 5.

there is, I think, no room for doubt that they, as well as the filamentous growths which bear the gemmae, are of gametophytic nature. Here then is a further instance of apospory; again it occurs in a fern grown in a close damp atmosphere and in shade, these being the conditions under which certain other examples have appeared. Nor is the apospory thus shown to occur in *Trichomanes* to be regarded as a mere sport, or so very rare and isolated a phenomenon, for we now see *Tr. Kaulfussii* from two separate collections showing the aposporous development profusely. The same was the case with *Tr. alatum*, a closely allied species, which was aposporous both in

the Edinburgh and Kew collections. It would appear probable that it is a peculiarity which may be induced, or at least its further development promoted, in certain species by particular conditions of culture: it is to be remembered, however, that it is not readily induced by moist culture in ordinary ferns, as I have shown by experiment[1], and in ferns at large it is certainly of rare occurrence. Since I know of no reference to this abnormality in systematic books, it would appear to be uncommon or even absent in the specimens of *Trichomanes* from their native habitat, upon which systematic writers will have based their descriptions.

In this, as in other cases of apospory, it is difficult to define the exact limit of the parts representing the two generations: examining them externally, the form and nature of the appendages (rhizoids, or sexual organs) and of the constituent cells have been used as diagnostic characters; it is possible, however, that the constitution of the nucleus may come to be recognized as a strict diagnostic character. If the generalization be correct, that the nuclei of the gametophyte on division show only half the number of chromosomes shown by those of the sporophyte, then clearly the cells in which the reduction takes place will be those which will define the limit between the generations; on this point detailed observations will be awaited with peculiar interest.

F. O. BOWER, Glasgow.

Oct. 1894.

ON THE ASCENT OF SAP[2].—By Henry H. Dixon, B.A., Assistant to the Professor of Botany, Trinity College, Dublin, and J. Joly, M.A., Sc.D., F.R.S. Strasburger's experiments have eliminated the direct action of living protoplasm from the problem of the ascent of sap, and have left only the tracheal tissue, as an organized structure, and the transpiration-activity of the leaf wherein to seek an explanation of the phenomenon. The authors investigate the capability of the leaf to transpire against excessive atmospheric pressures. In these experiments the leaf was found able to bring forward its water meniscuses against the highest pressures attained and freely transpire. Whether the draught upon the sap established at the leaf during transpiration be regarded as purely capillary or not, these

[1] Annals of Botany, Vol. iv. p. 168.

[2] Abstract of a paper read before the Royal Society, November 15, 1894.

experiments lead the authors to believe that it alone is quite adequate to effect the elevation by direct tension of the sap in tall trees. Explanations of the lifting of the sap from other causes prove inadequate.

A reconsideration of the principal experiments of previous observers and some new experiments of the authors lead to the view that the ascent is principally in the lumen and not in the wall.

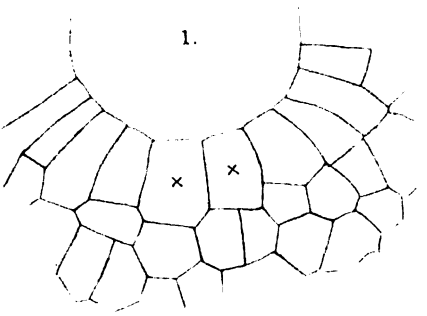
The explanation of how the tensile stress is transmitted in the ascending sap without rupture of the column of liquid is found in the stable condition of this liquid. The state of stability arises from two circumstances:—the internal stability of a liquid when mechanically stretched, whether containing dissolved gases or not, and the additional stability conferred by the minutely subdivided structure of the conducting tissue, which renders the stressed liquid stable even in the presence of free gas.

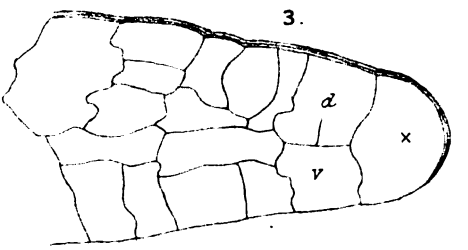
By direct experiments upon water containing large quantities of dissolved air, the state of internal stability is investigated. And, further, by sealing up in the vessels, in which the water to be put under tension is contained, chips of the wood of *Taxus baccata*, the authors find that their presence in no case gives rise to rupture of the stressed liquid, but that this occurs preferably anywhere else, and usually on the glass walls. The establishment of tensile stress is effected in the usual way, by cooling the completely filled vessel. A measurement possessing considerable accuracy afforded 7½ atmospheres as being attained in some of the experiments.

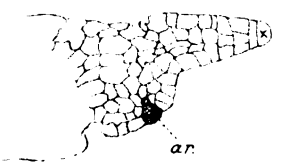
The second condition of stability arises directly from the property of the pit-membranes to oppose the passage of free gas, while they are freely permeable to the motion of a liquid. Hence a chance development of free gas is confined in effect to the minute dimensions of the compartment in which it is evolved, and this one lumen alone is rendered for the time being non-conducting. On the other hand, in the water-filled portion of the tracheal tissue, the closing membranes, occupying the median and least obstructive position, the motion of the stressed sap is freely allowed. The structure of the conducting tissue is, in fact, a configuration conferring stability on a stressed liquid in the presence (from various causes) of free gas. As neither free gas nor unwetted dust particles can ascend with the sap, the authors contend that the state of tensile stress necessary to their hypothesis is inevitably induced.

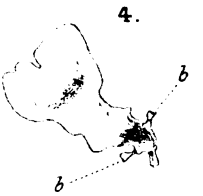
The energy relations of the leaf with its surroundings, on the assumption that evaporation at capillary water-surfaces is mainly responsible for the elevation of sap, may be illustrated by the well-known power of the water-filled porous pot to draw up mercury in a tube to which it is sealed. The authors describe an engine in which the energy entering in the form of heat at the capillary surfaces may be in part utilized to do mechanical work: a battery of twelve small porous pots, freely exposed to the air, keeping up the continuous rotation of a fly-wheel. Replacing the porous pots by a transpiring branch, this too maintains the wheel in rotation. This is, in fact, a vegetable engine. In short, the transpiration effects going on at the leaf are, in so far as they are the result of spontaneous evaporation and uninfluenced by other physiological phenomena, of the 'sorting demon' class, in which the evaporating surface plays the part of a sink of thermal energy.

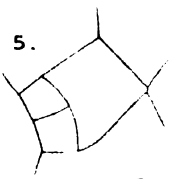
If the tensile stress in the sap is transmitted to the root, the authors suggest that this will establish in the capillaries of the root-surface meniscuses competent to condense water rapidly from the surrounding soil. They show by experiment the power possessed even by a root injured by lifting from the soil, of condensing water vapour from a damp atmosphere. Such a state of things may be illustrated by a system (which the authors realised) consisting of two porous pots connected by a tube and all filled with water; one, the 'leaf,' exposed to the air gives out vapour, the other, the 'root,' buried in damp earth supplies the demand of the 'leaf,' and an upward current in the connecting tube is established.

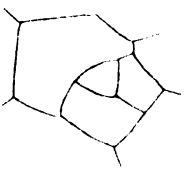


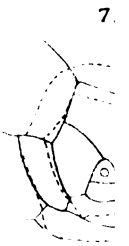


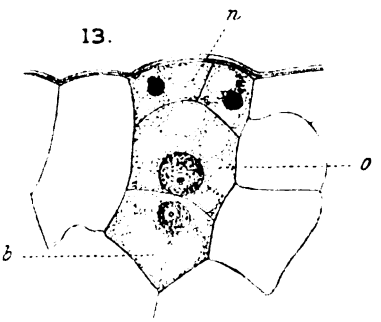


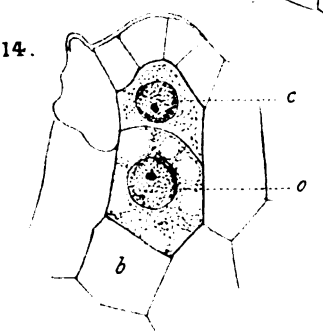


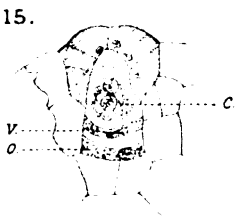


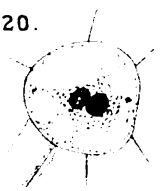


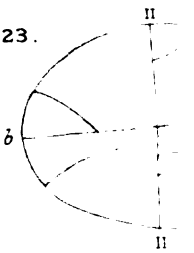


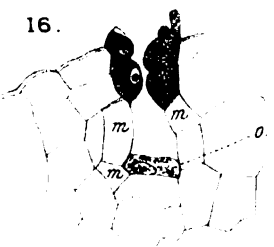


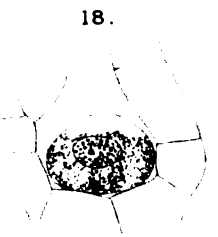


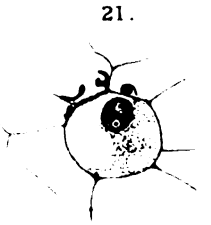












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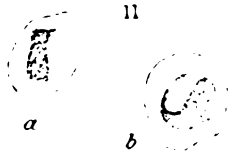

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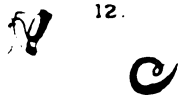

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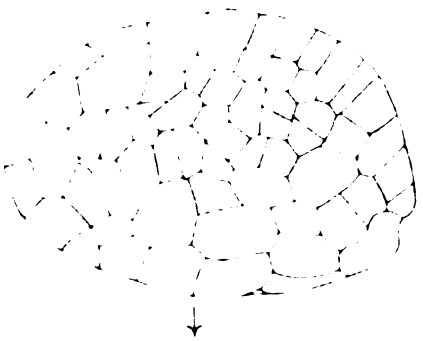

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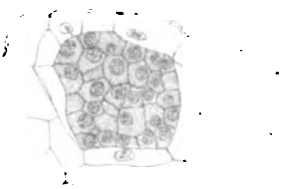

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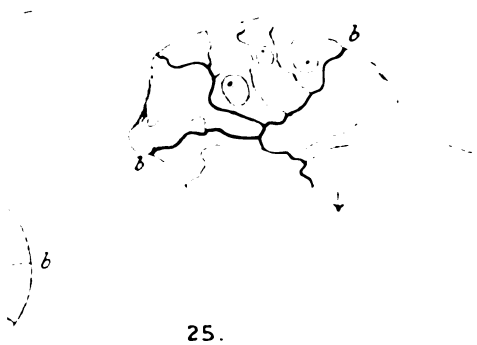

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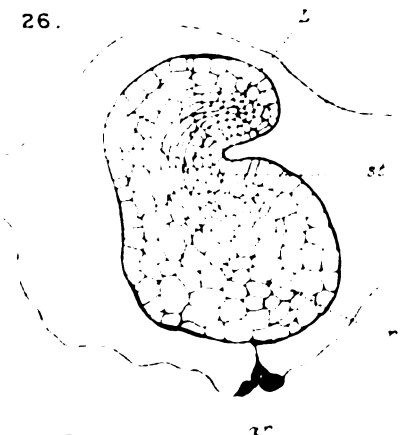

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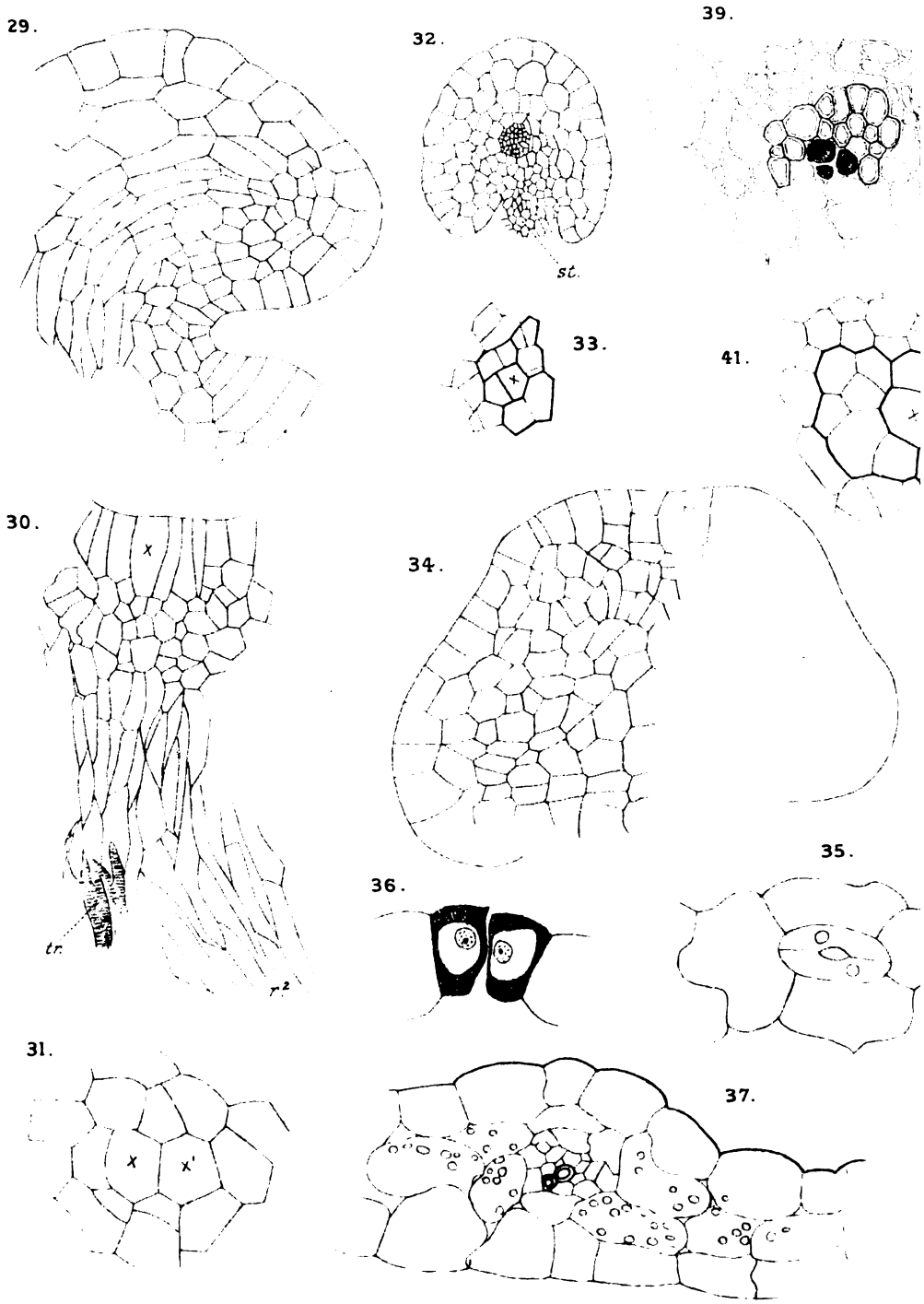

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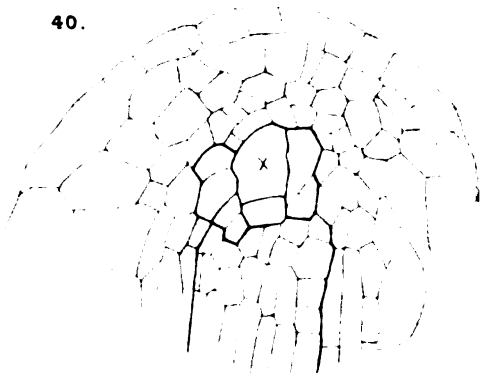

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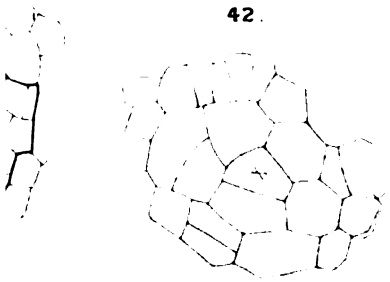

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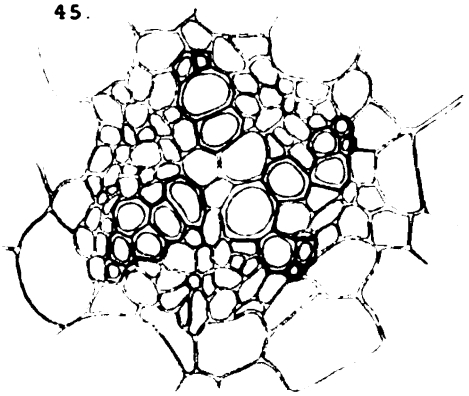

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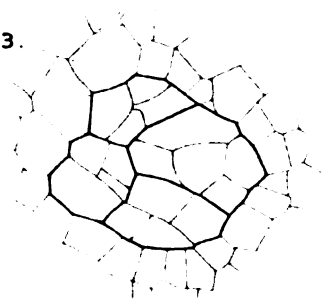

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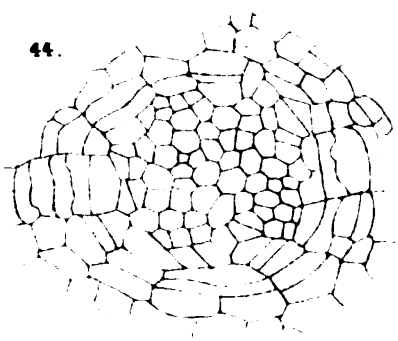

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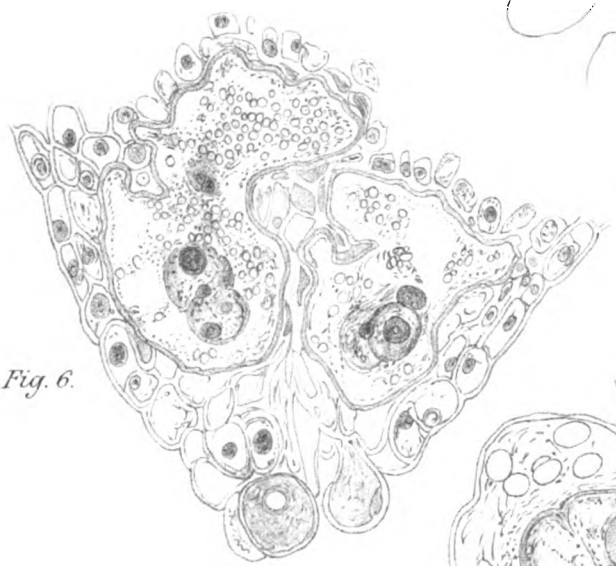

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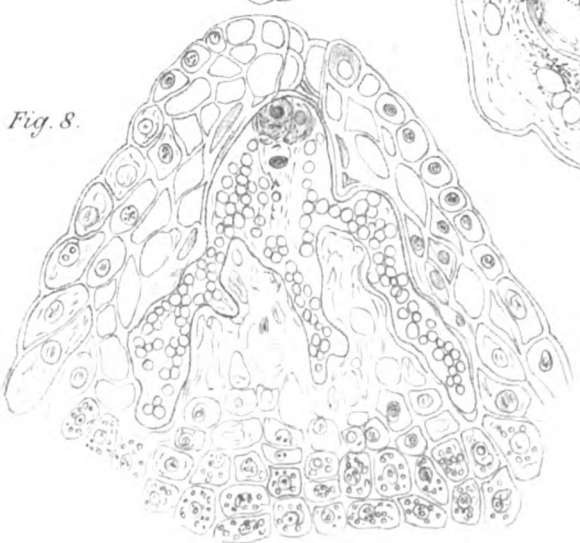

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DIXON. — ON FERTILISATION OF PINUS.

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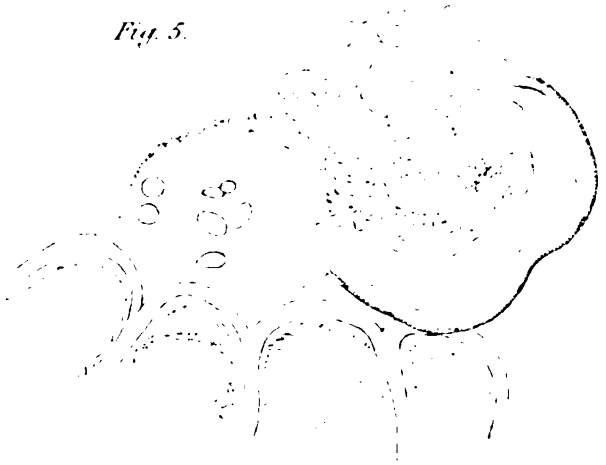

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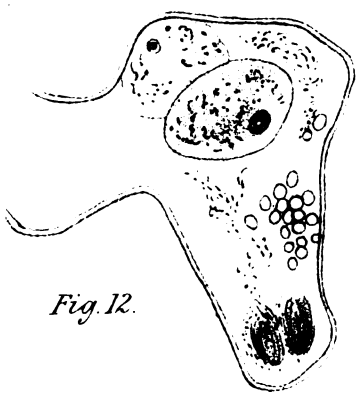

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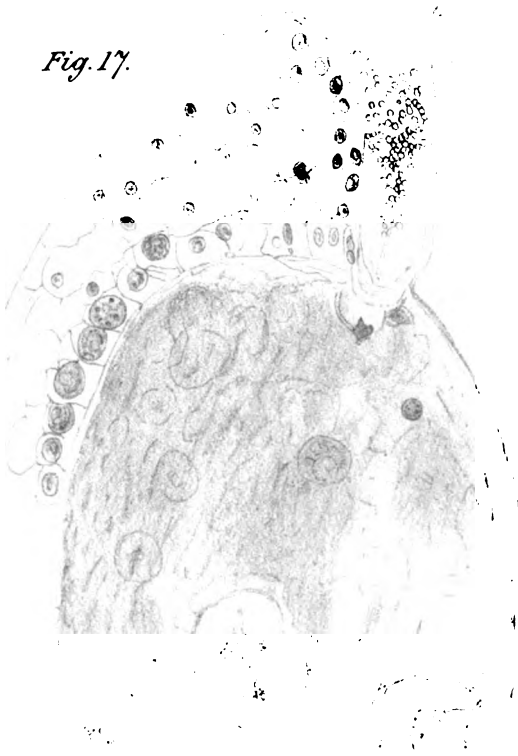

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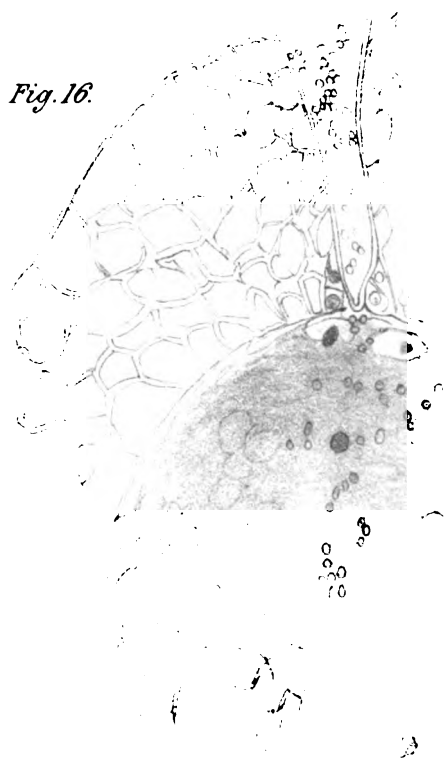

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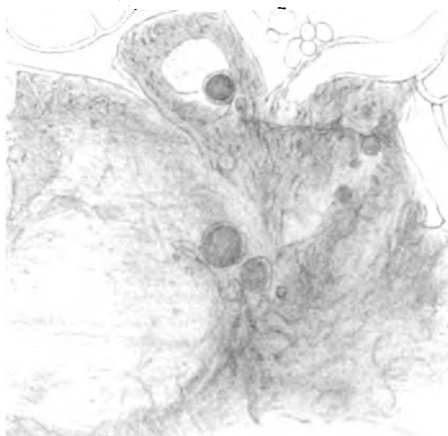

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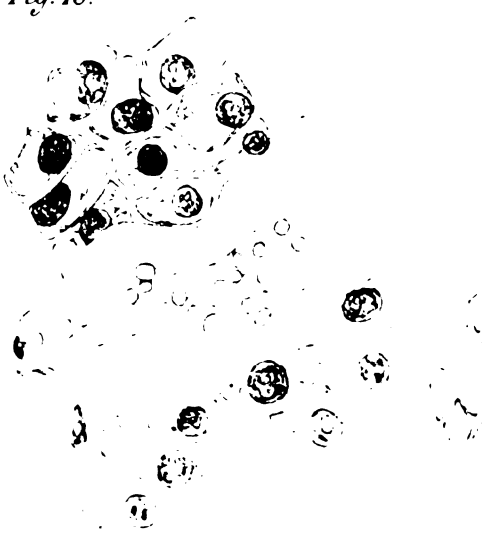

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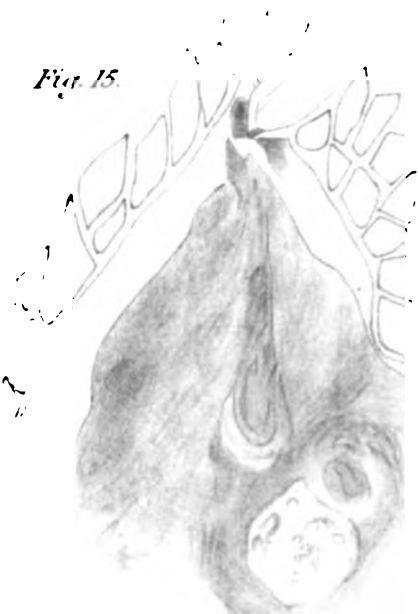

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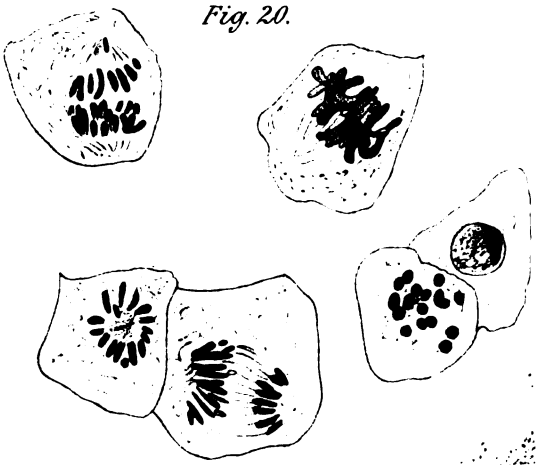

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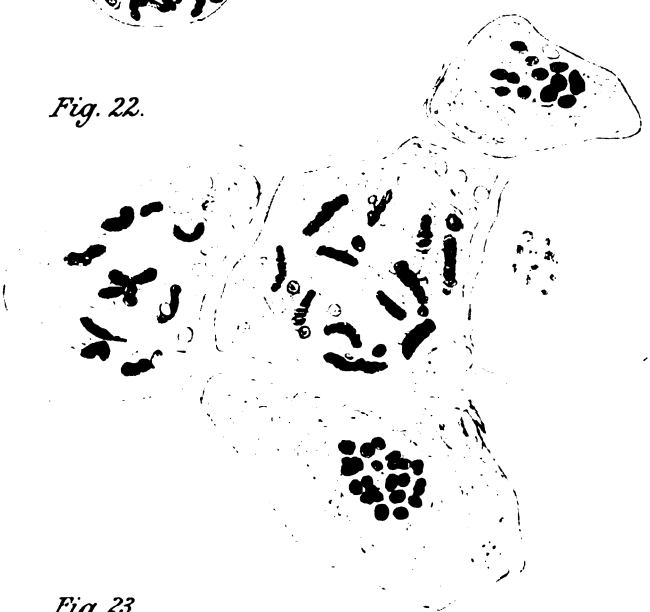

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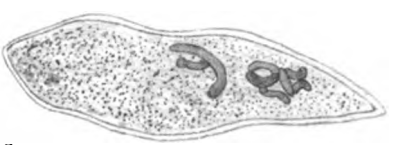

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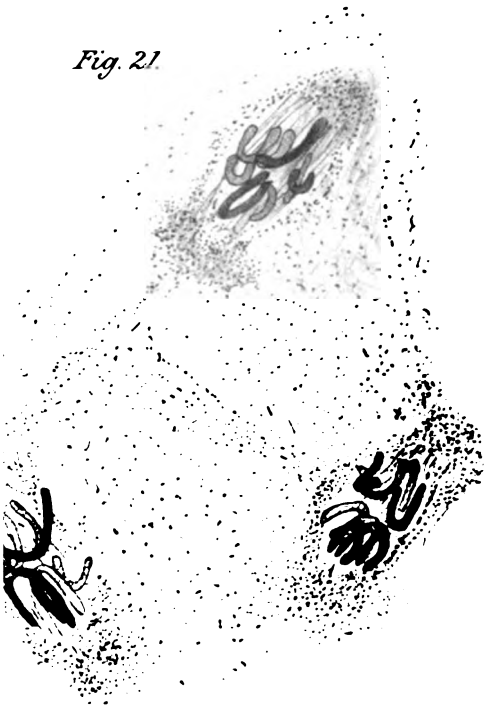

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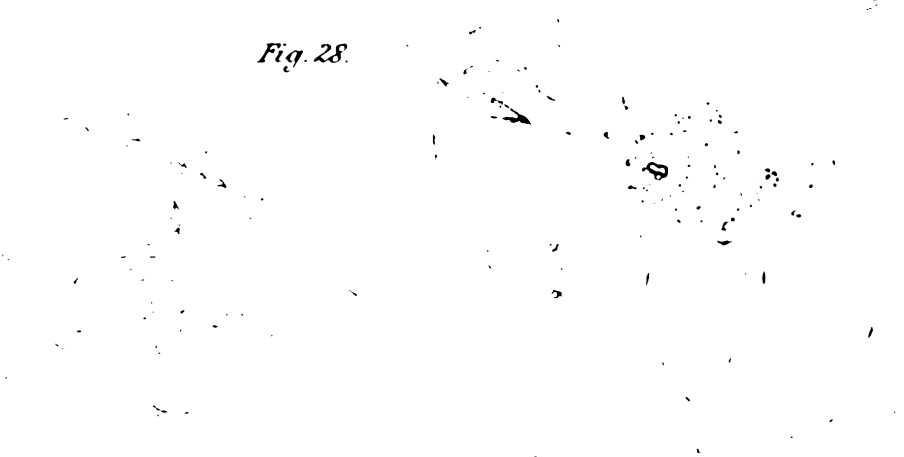

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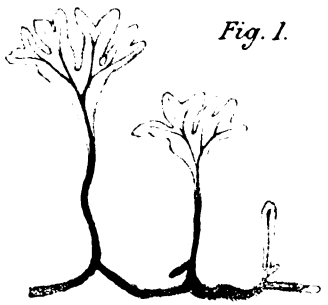

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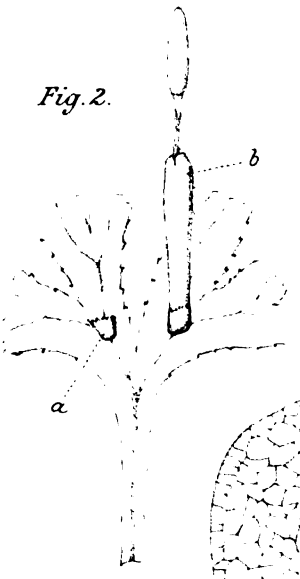

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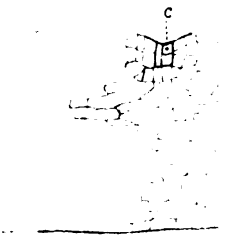

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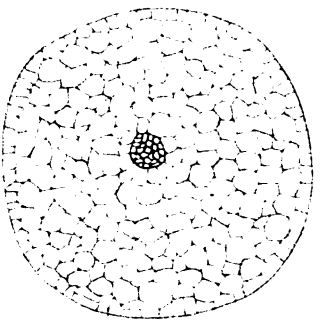

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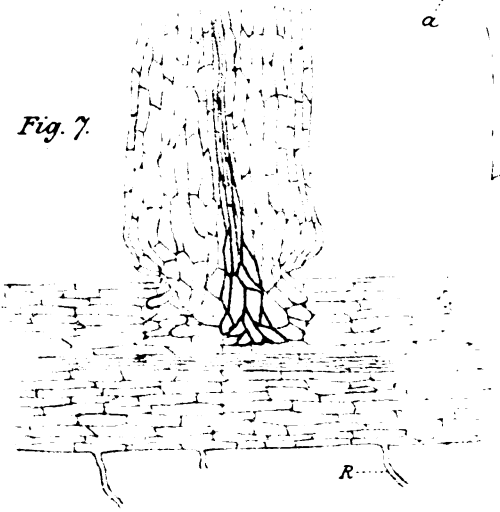

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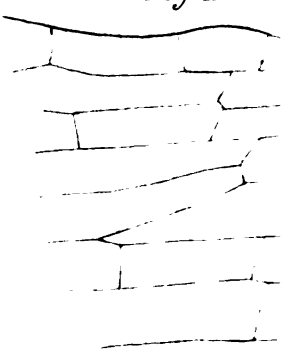
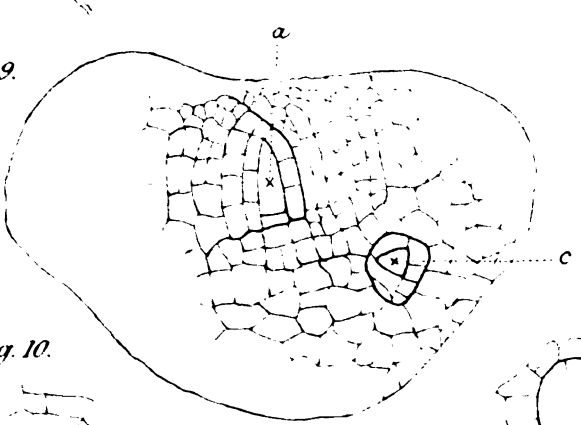


Fig. 11.



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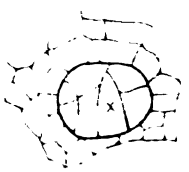

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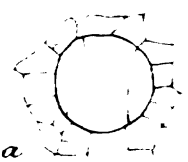

Fig. 10a

Fig. 12

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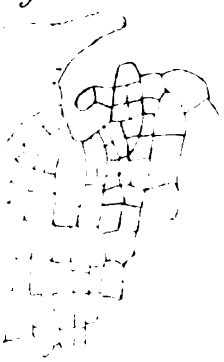
Fig. 4.

Fig. 5.

Fig. 6.

Fig. 14.



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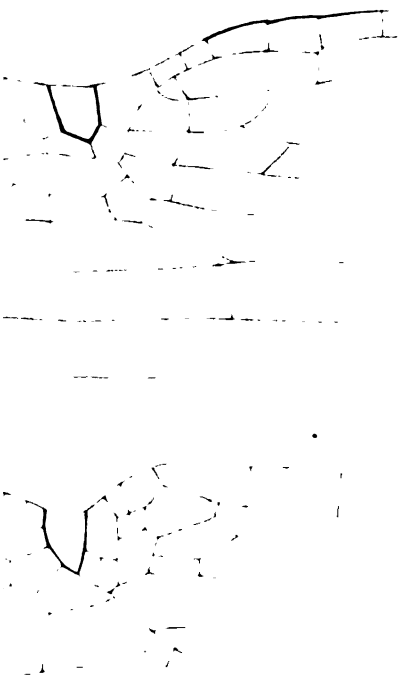

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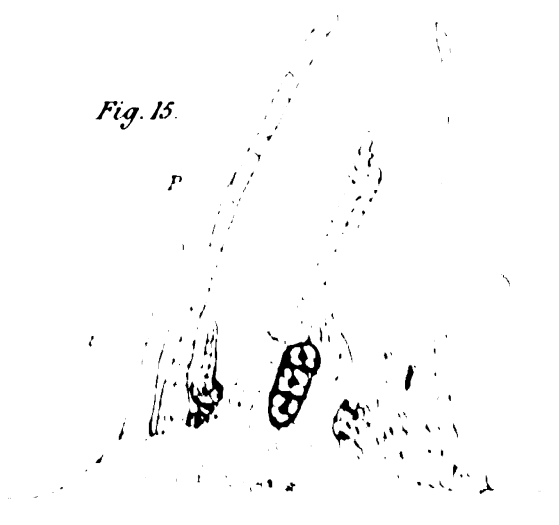

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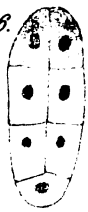

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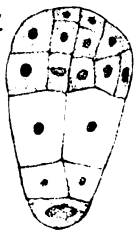

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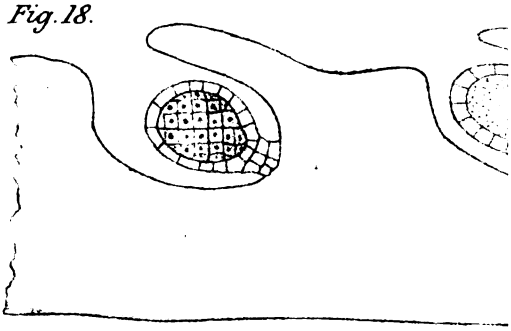

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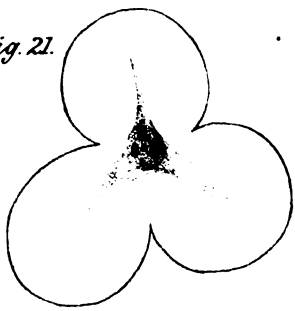

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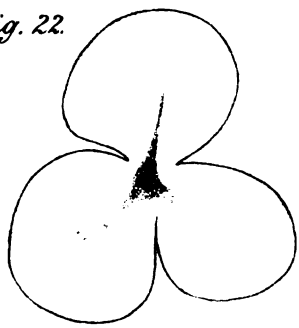
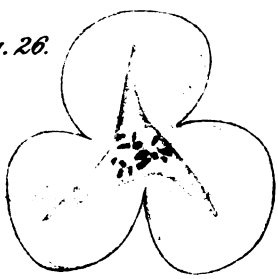


Fig. 23.

Fig. 26.



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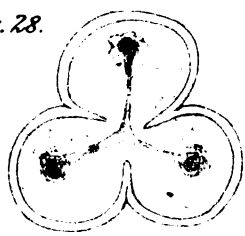

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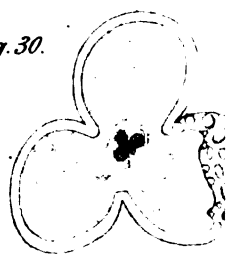

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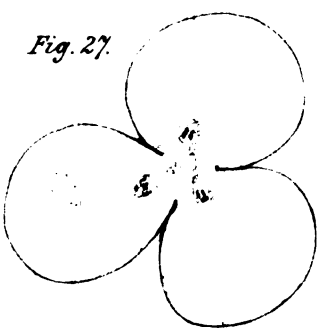

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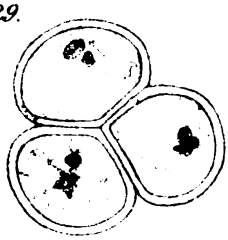

Fig. 31.

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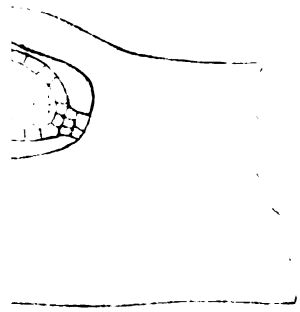

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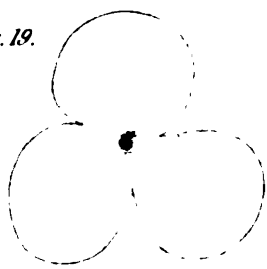

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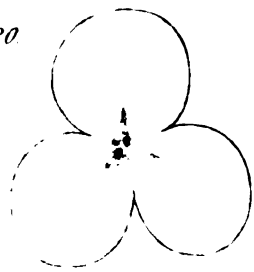

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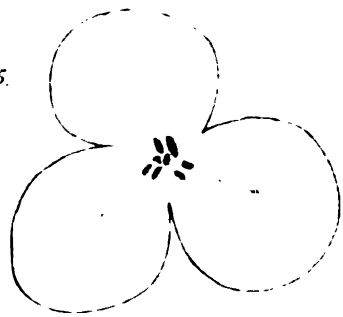


Fig. 32.

Fig. 34.

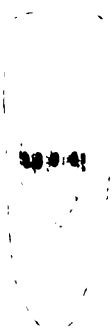
Fig. 35

Fig. 36.

Fig. 37.

Fig. 37a

33.



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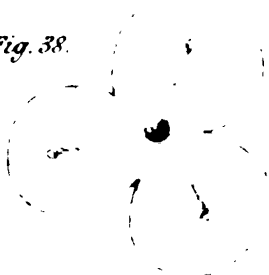

Fig. 39

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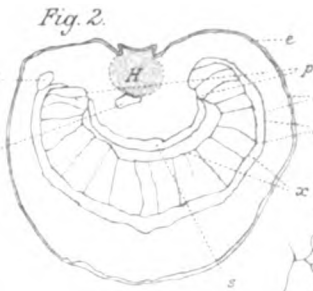

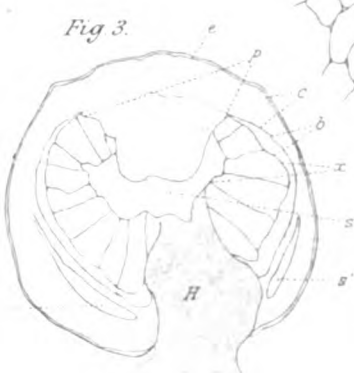

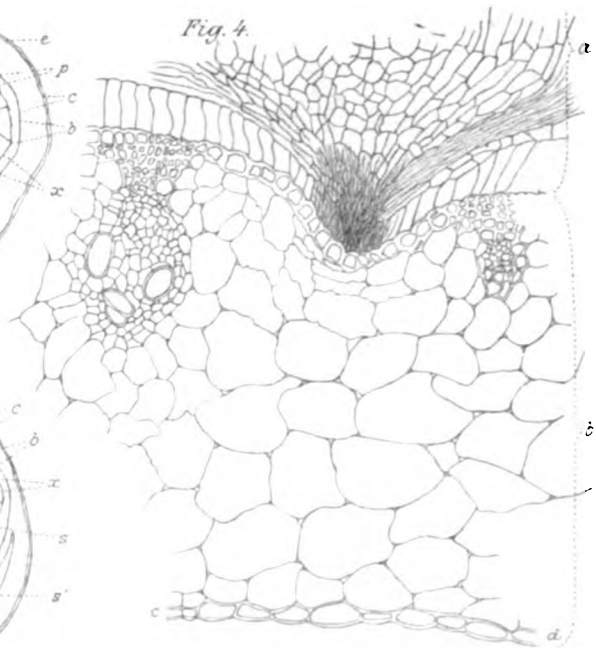

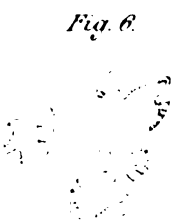

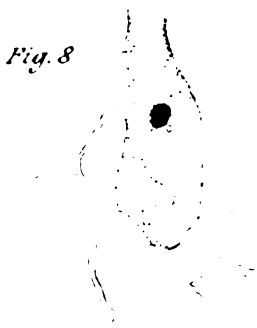

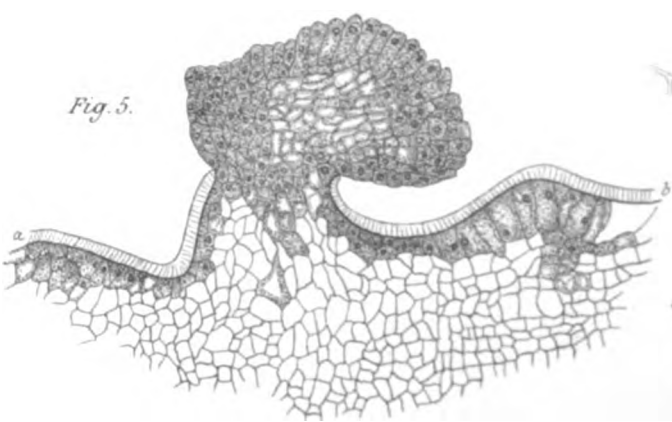

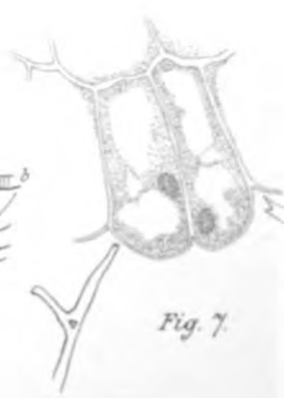

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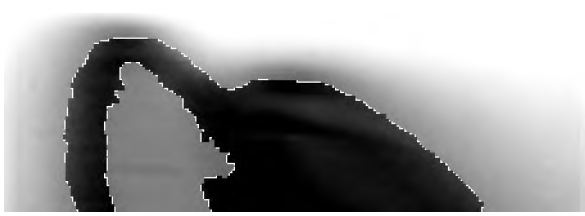
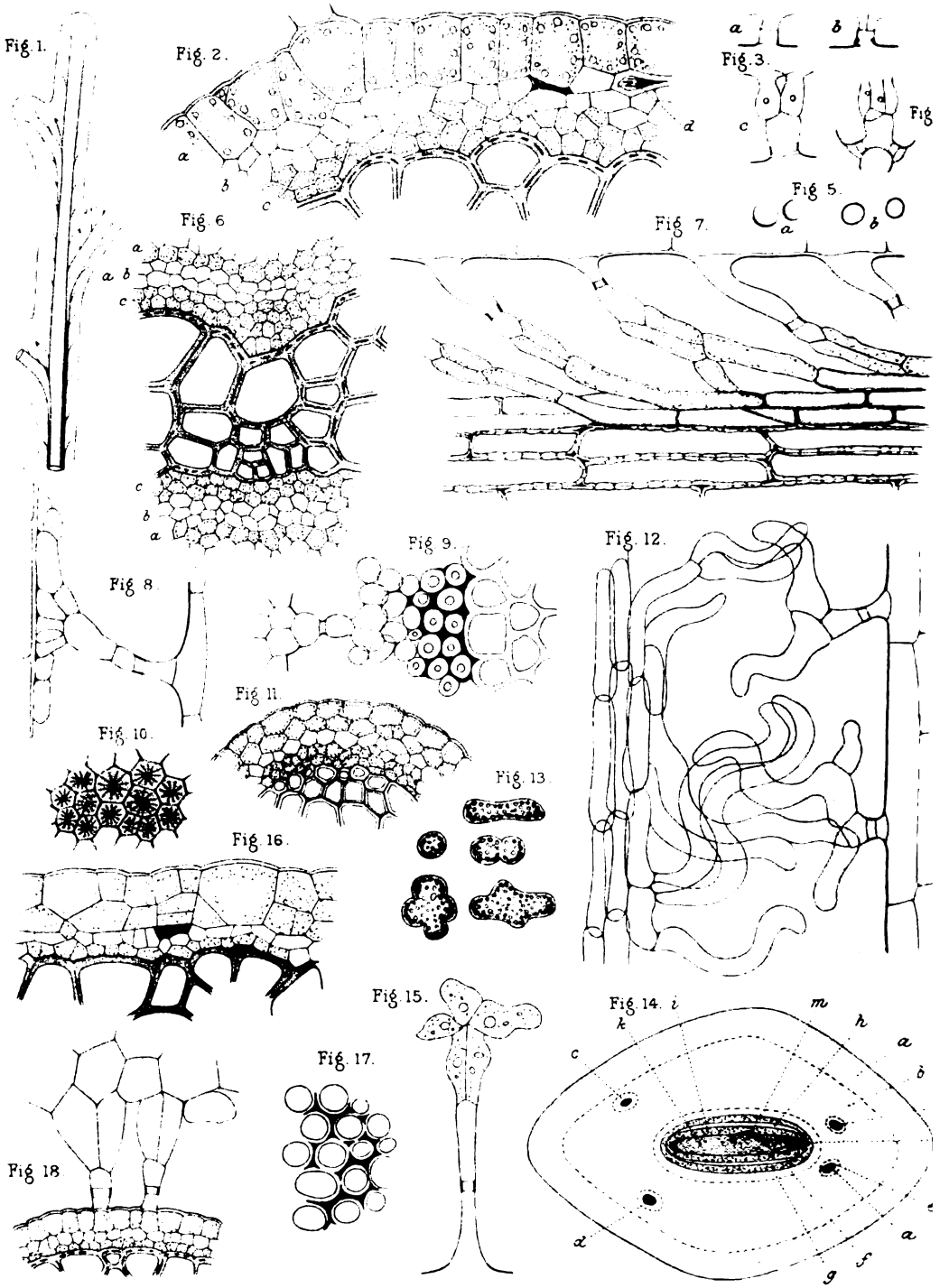
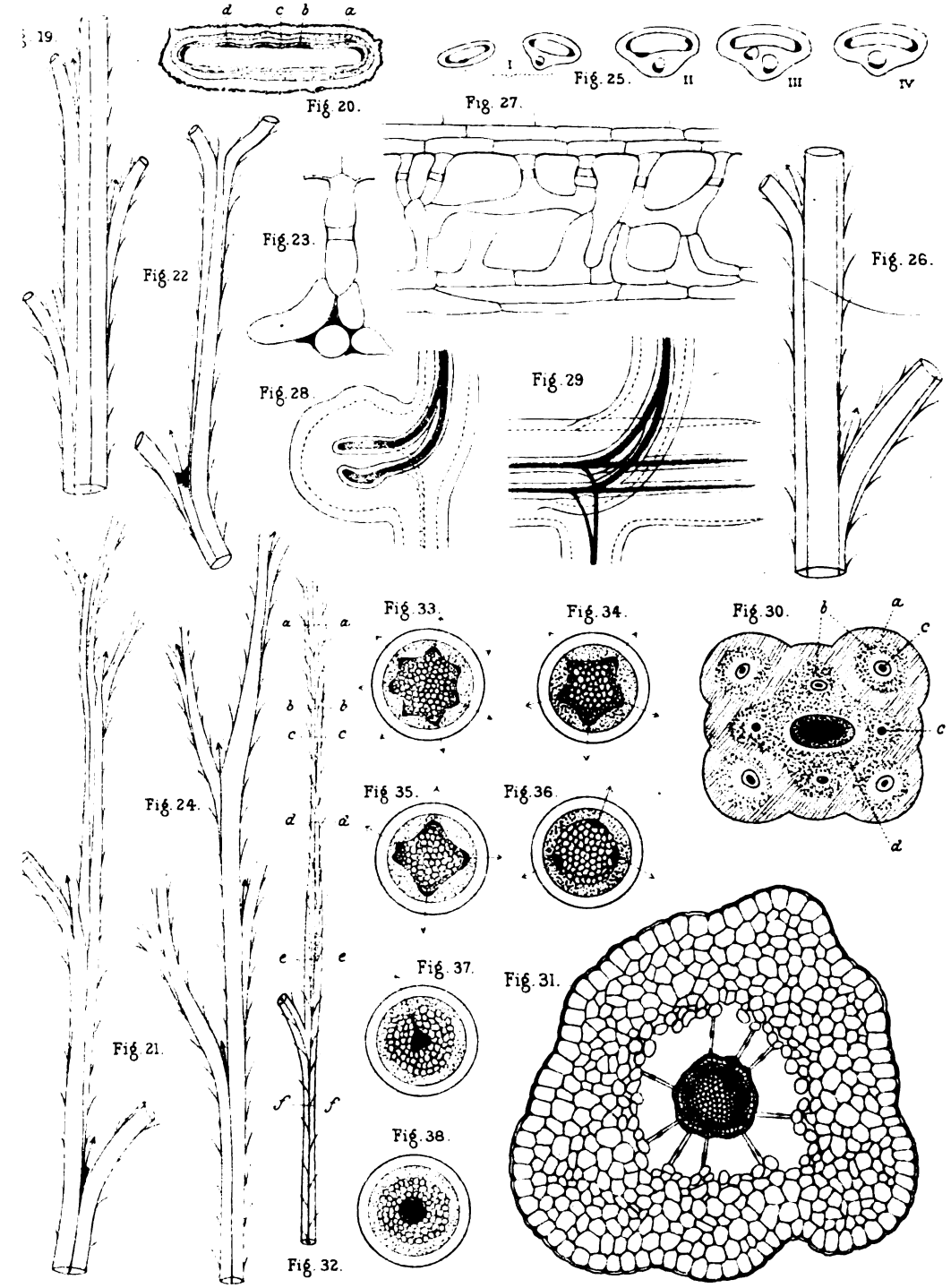




HARVEY GIBSON. — Figs 1-5, SELAGINELLA MARTENSII, Spr.; Figs 6,7, S. GRANDIS, Mr.; Fig. 8, S. VOGELII, Spr.; Fig. 9, S. HÆMATODES, Spr.; Fig. 10, S. ERYTHROPUS, Spr.; Fig. 11, S. GRIFFITHII, Spr.; Figs 12,13, S. VITICULOSA, Klot.; Figs 14,15, S. SERPENS, Spr.; Fig. 16, S. MOLLICEPS, Spr.; Fig. 17, S. INVOLVENS, Spr.; Fig. 18, S. PATULA, Spr..



Figs 19, 20, S. FLABELLATA, Spr.; Fig. 21, S. ATROVIRIDIS, Spr.; Figs 22, 23, S. BAKERIANA, Bl.; Figs 24, 25, S. UNCINATA, Spr.; Figs 26-29, S. BRAUNII, Bak.; Fig. 30, S. OREGANA, Eat.; Figs 31-38, S. SPINOSA, P.B.

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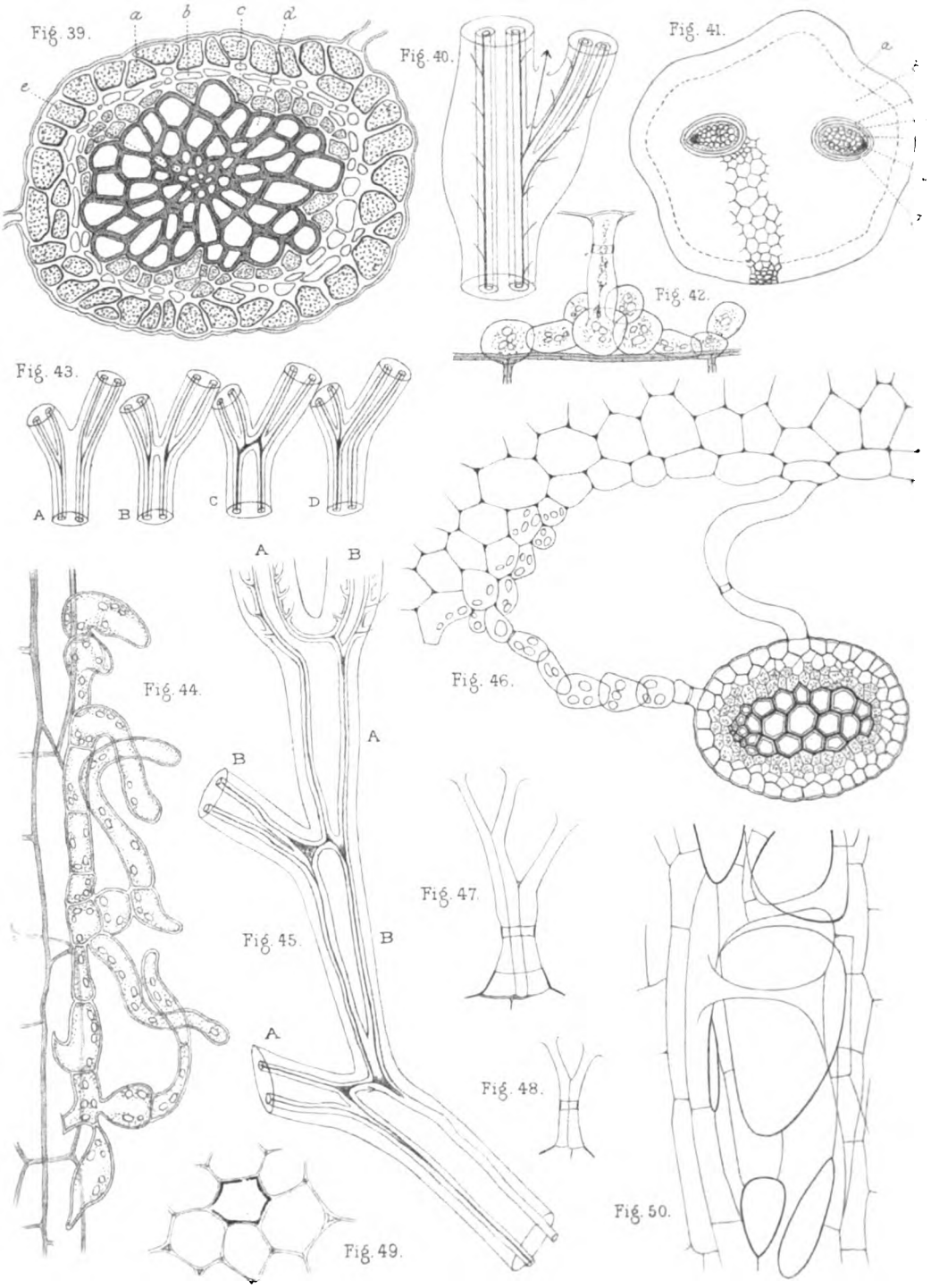
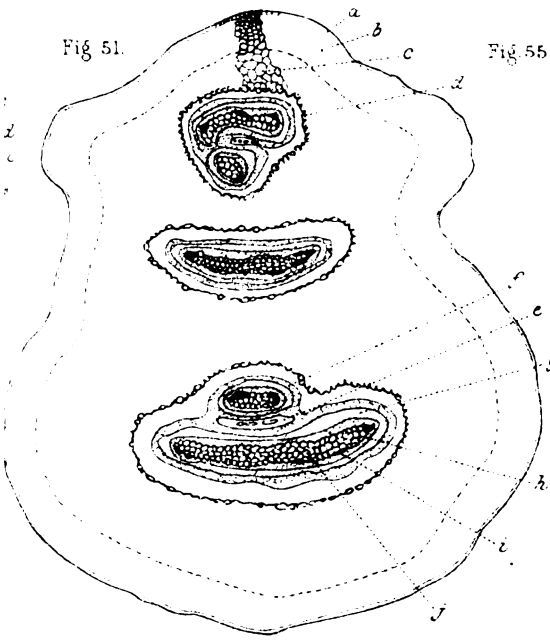
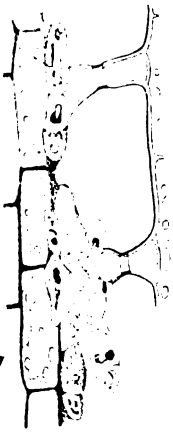
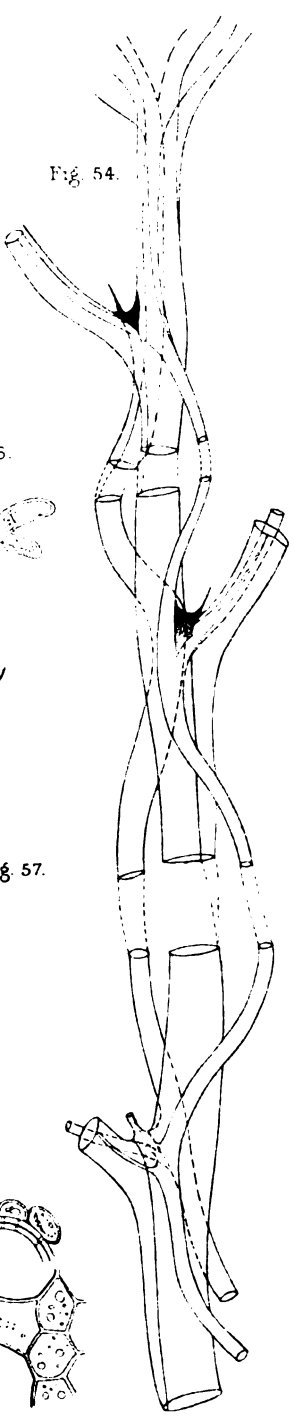
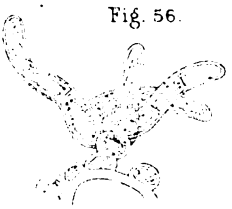


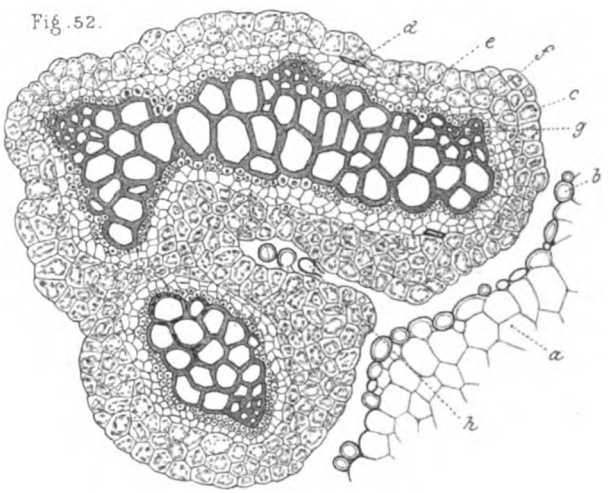
HARVEY GIBSON. Fig. 39, SELAGINELLA SPINOSA, P.B.; Figs 40-42, S. GALEOTTEI, Spr.; Fig. 43, S. DELICATISSIMA, A.Br.; Fig. 44, S. SULCATA, Spr.; Figs 45-48, 50, S. KRAUSSIANA, A.Br.; Fig. 49, S. RUBELLA, Moore.

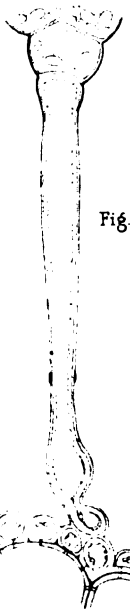


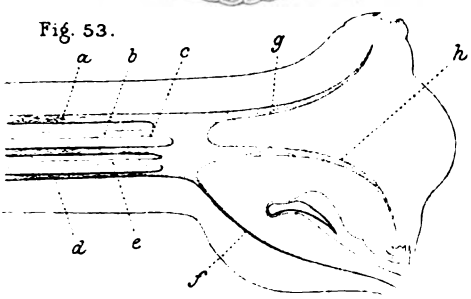


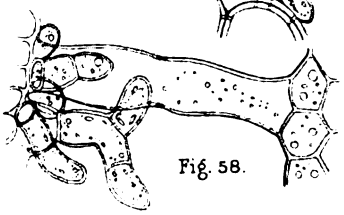












Figs 51–55, S. INÆQUALIFOLIA, Spr.; Figs 56–58, S. WALLICHII, Spr..

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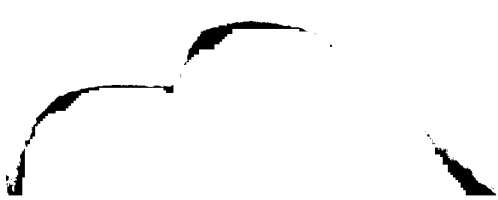
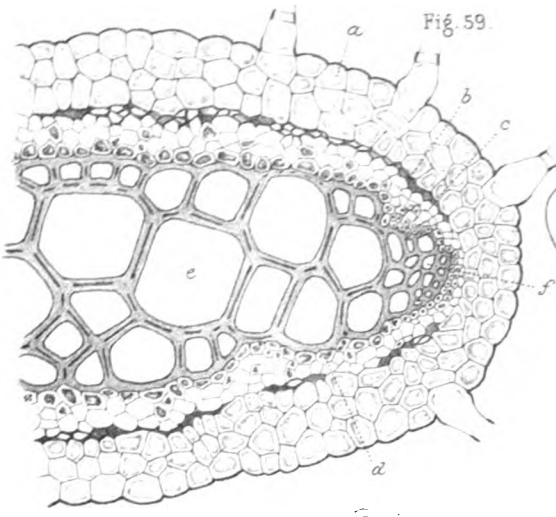
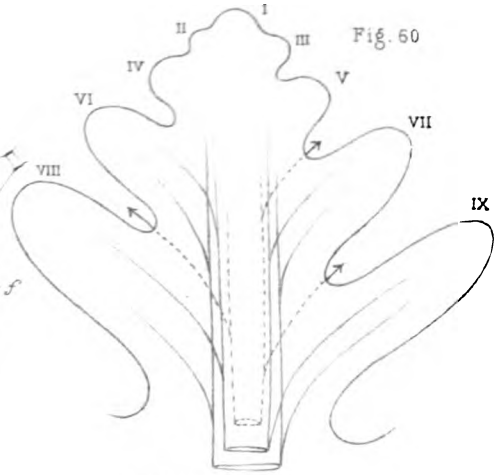
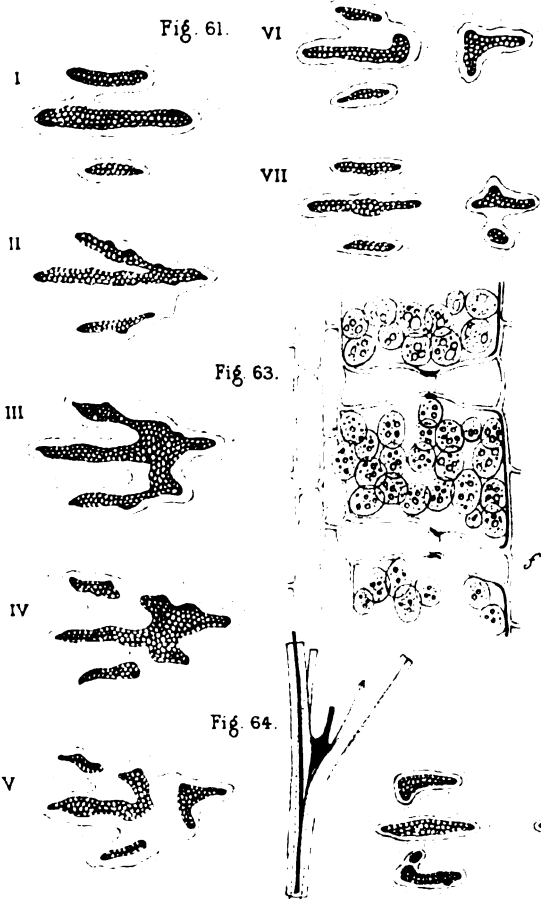
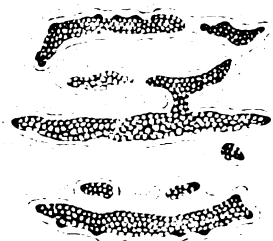
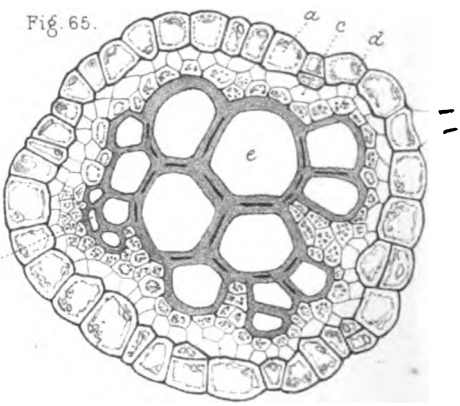


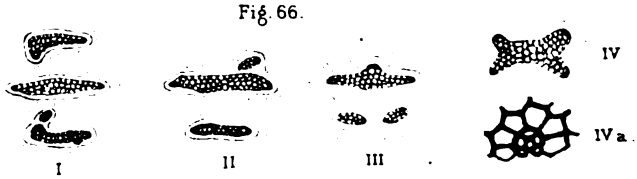






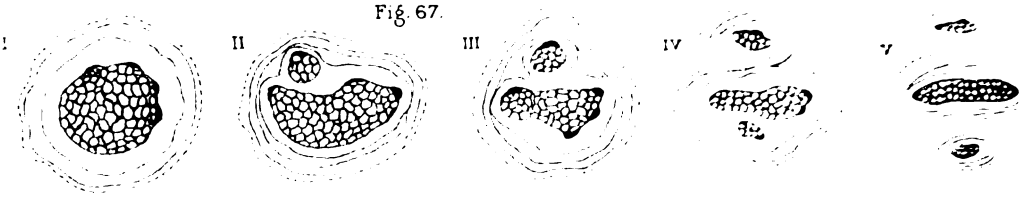






HARVEY GIBSON. — Figs 59,60, SELAGINELLA WALLICHII, Spr.; Figs 61,62, S. WILLDENOWII, Bak.; Fig. 63, S. CANALICULATA, Bak.; Figs 64,65, S. METTENII, A.Br.; Fig. 66, S. LOBBII, Moore.

Fig. 67.



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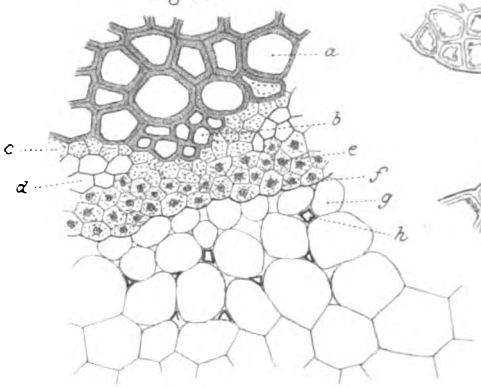

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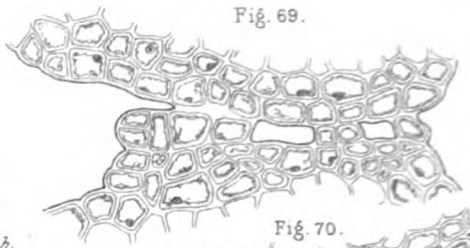
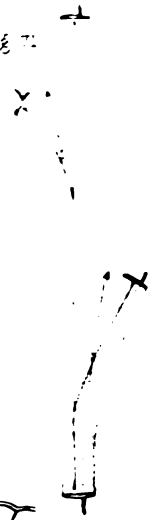


Fig. 70.

Fig. 71.



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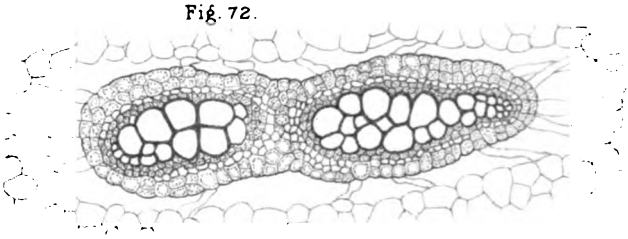

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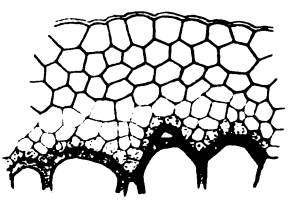
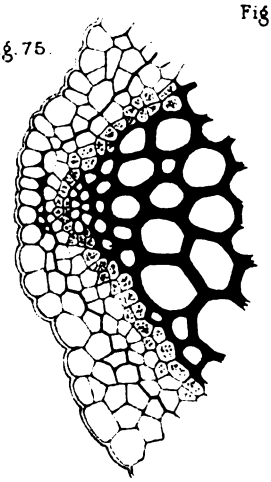


Fig. 73.

Fig. 75.



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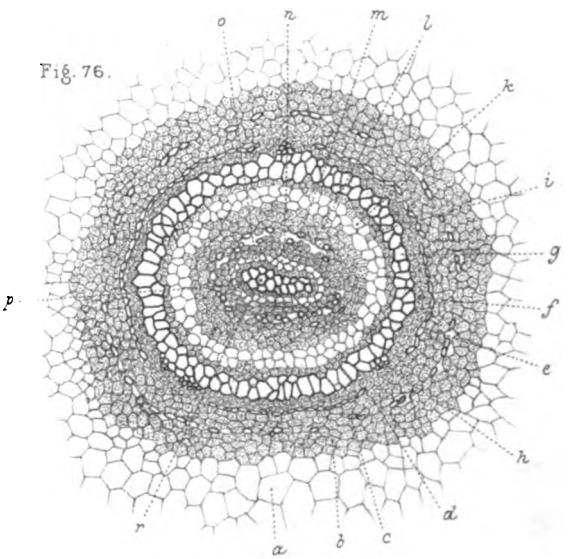

Figs 67–70, S. INÆQUALIFOLIA, Spr.; Figs 71, 72, S. VIRIDANGULA, Spr.; Fig. 73, S. CHILENSIS, Spr.; Fig. 74, S. LOBBII, Moore.; Fig. 75, S. CANALICULATA, Bak.; Fig. 76, S. LÆVIGATA, Bak. var. LYALLII, Spr.

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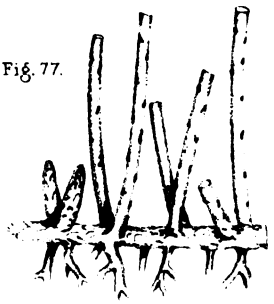
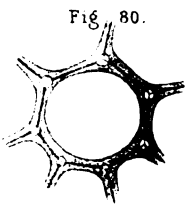


Fig. 77.

Fig. 79.



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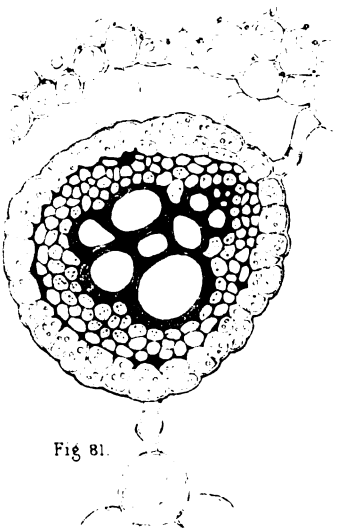

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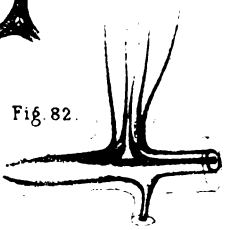

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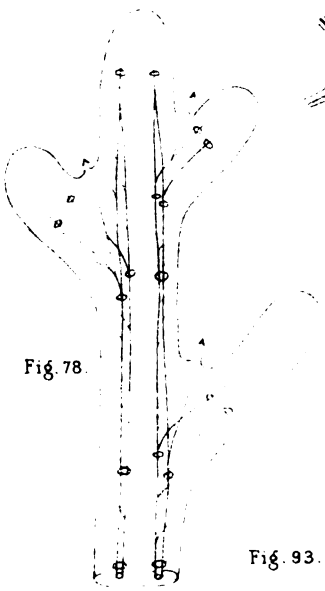

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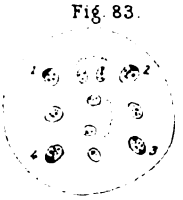

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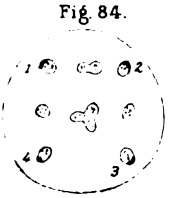

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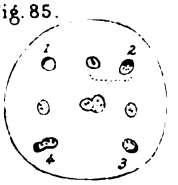

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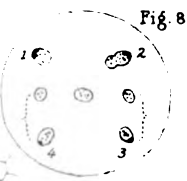

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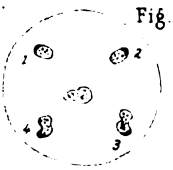

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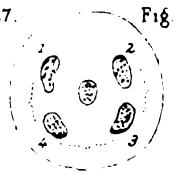

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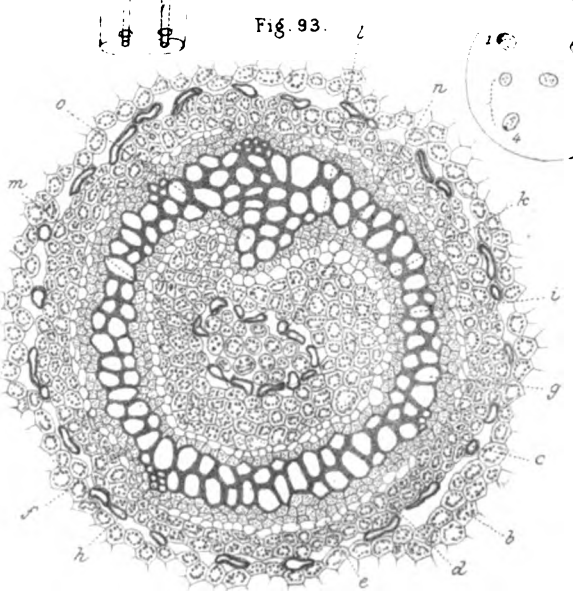
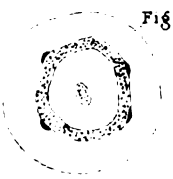


Fig. 93.

Fig. 89.



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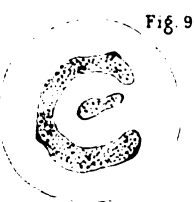

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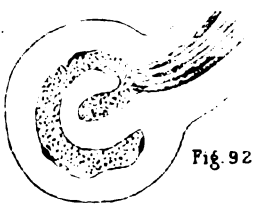
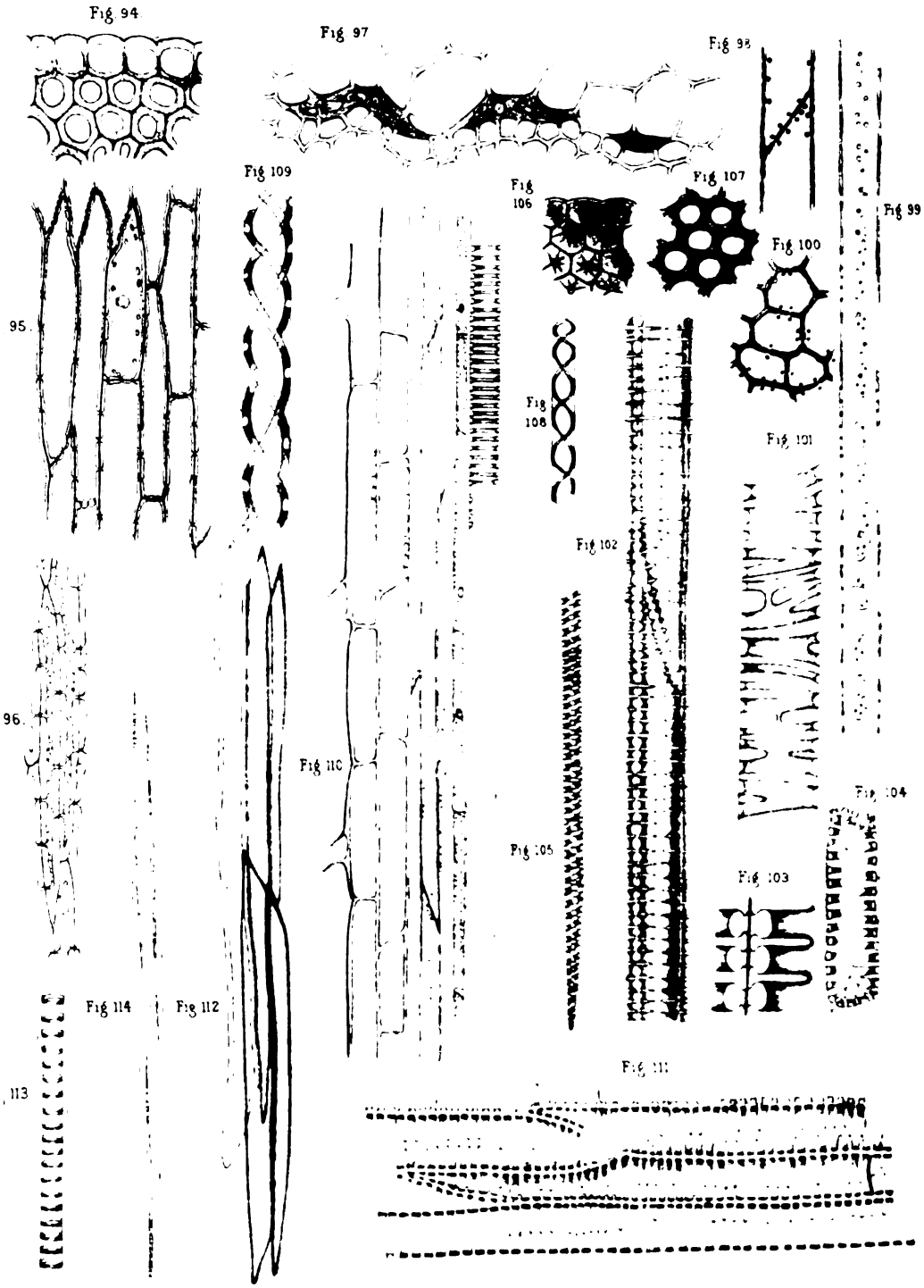


Fig. 92.

HARVEY GIBSON.— Figs 77-94, SELAGINELLA LÆVIGATA, Bak. var. LYALLII, Spr.



Figs 95 & 112, S. WALLICHII, Spr. Fig 96, S CAULESCENS, Spr. Figs 97–105, 113 & 114, S BRAUNII, Bak Figs 106, 107, S. LEPIDOPHYLLA, Spr., Figs 108 110, S. SULCATA, Spr. Fig 111, S. OREGANA, Eat

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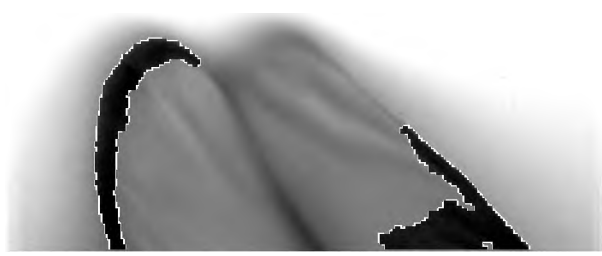

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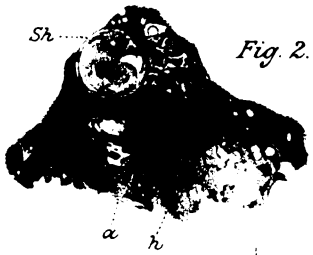
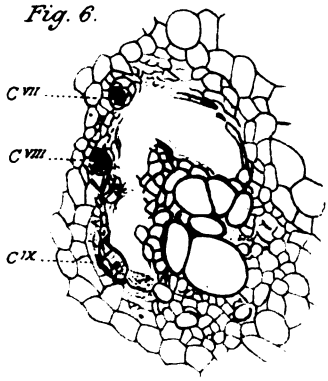


Fig. 2.

Fig. 4.

Fig. 6.



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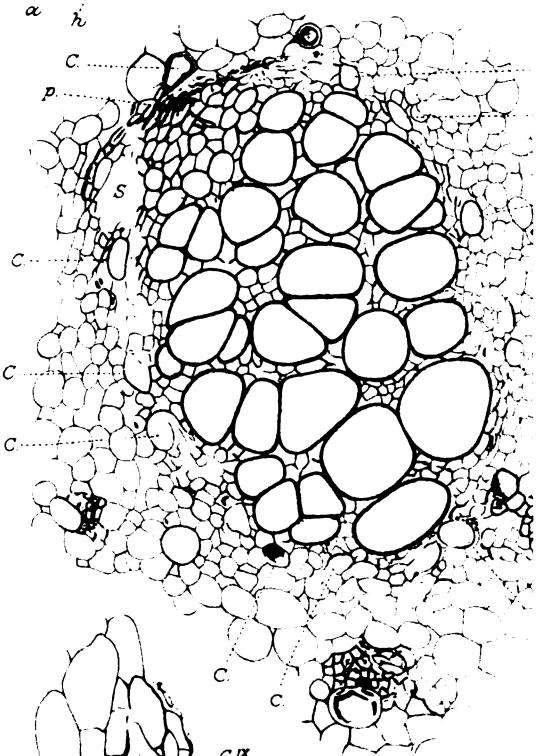

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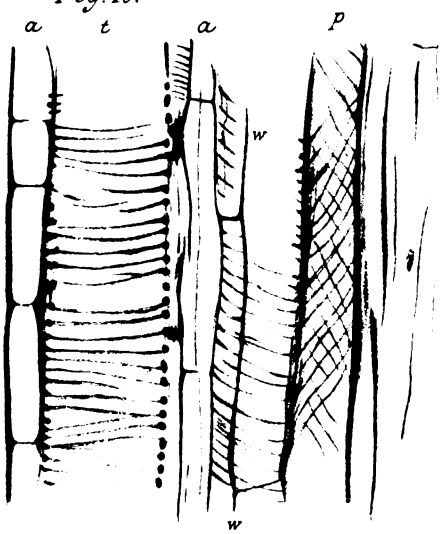

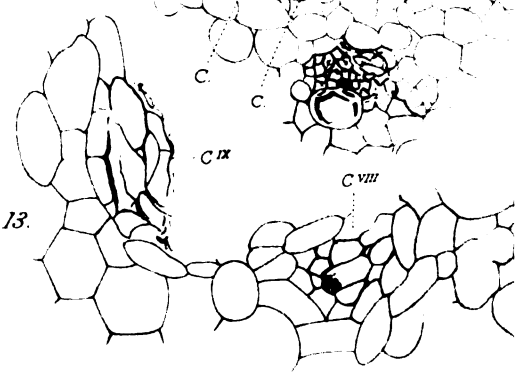

Fig. 13.

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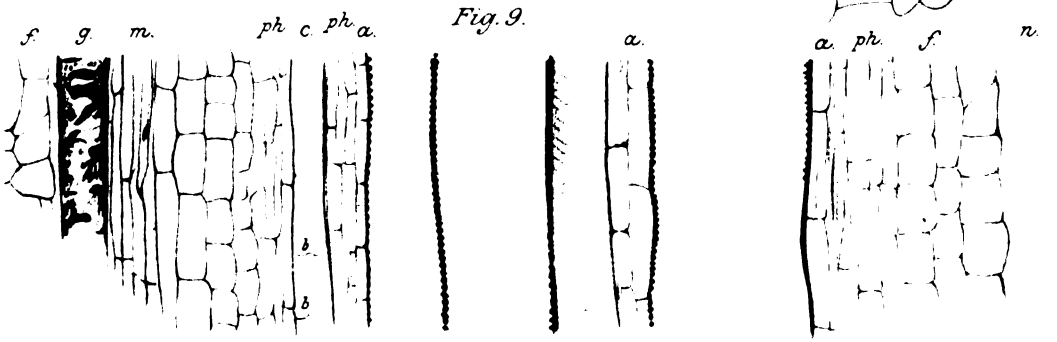

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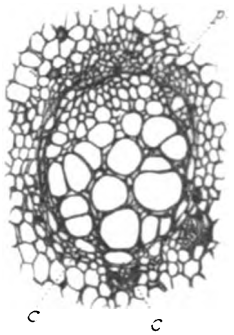

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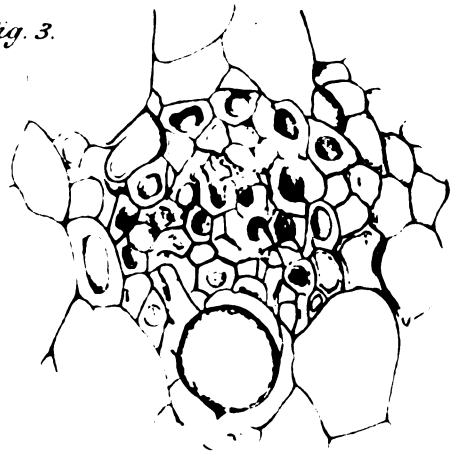

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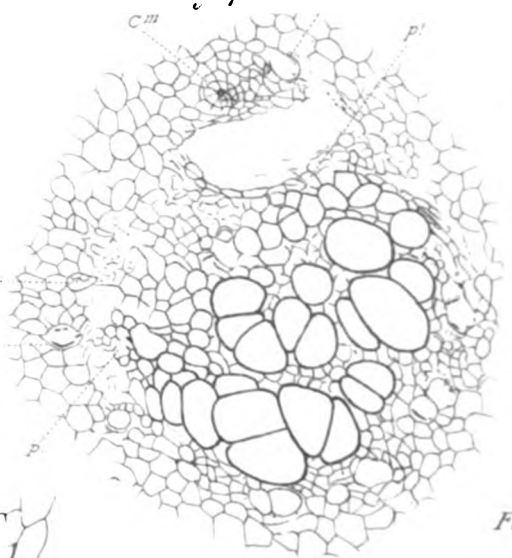

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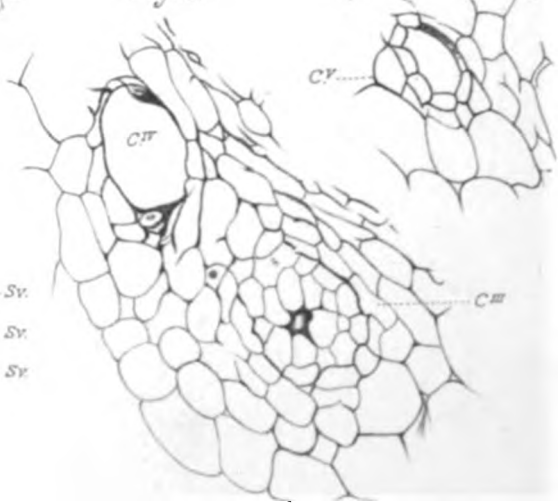

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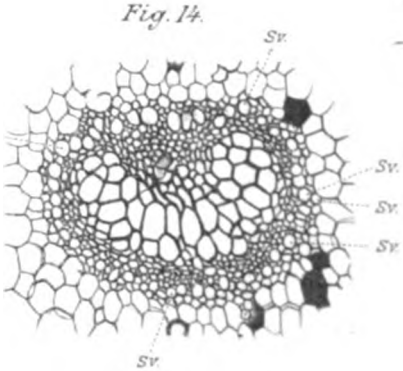

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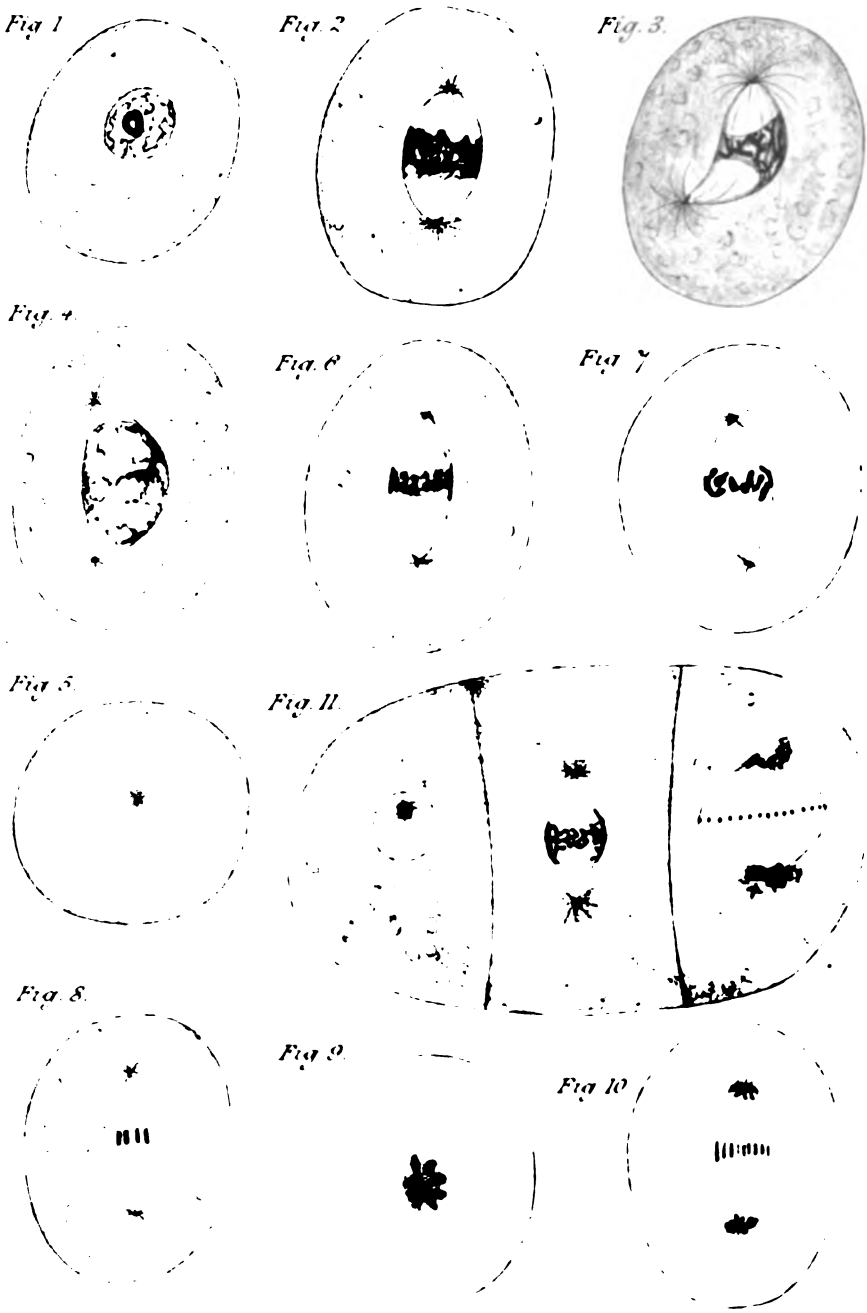

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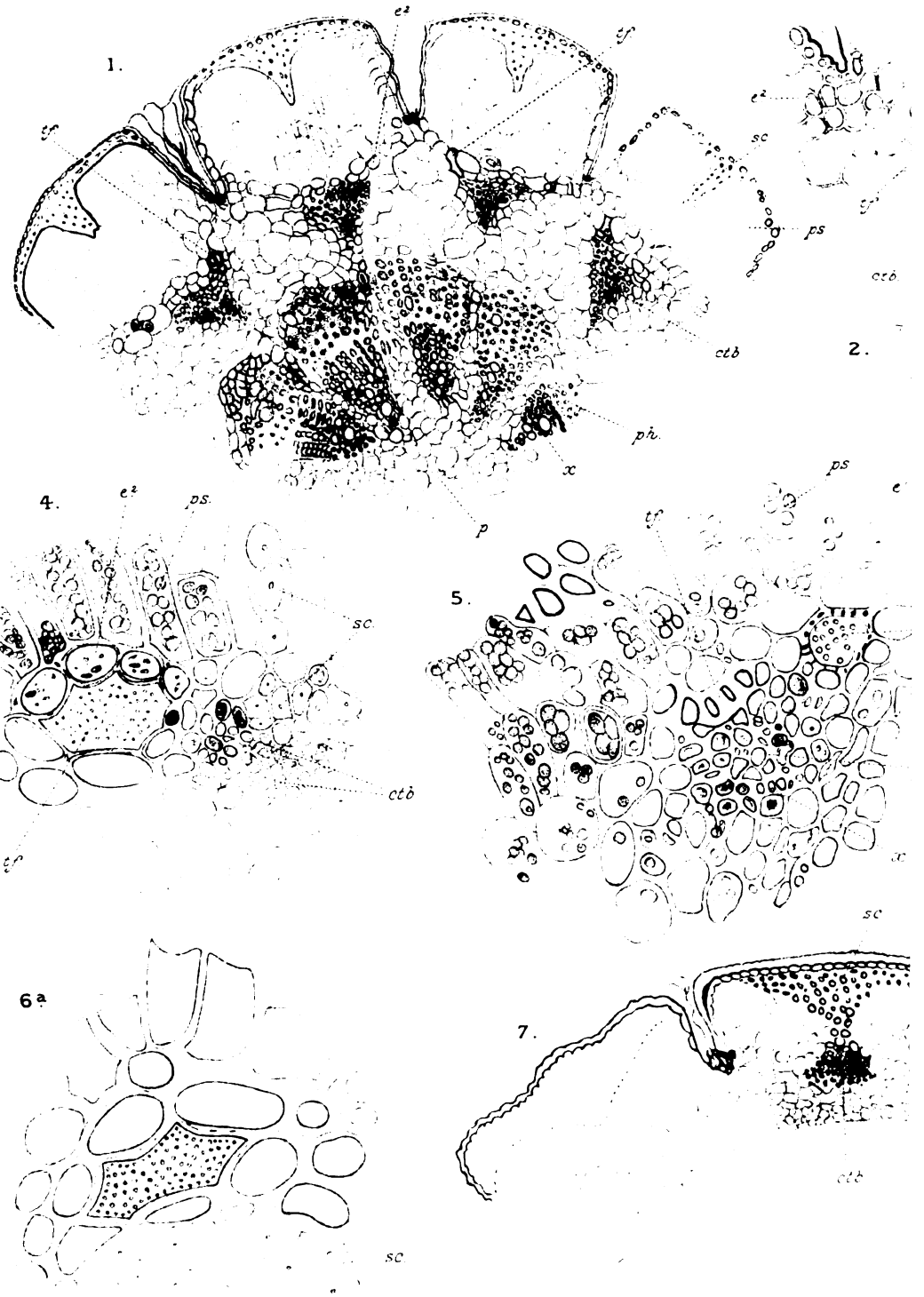
FARMER. — PELLIA EPIPHYLLA.



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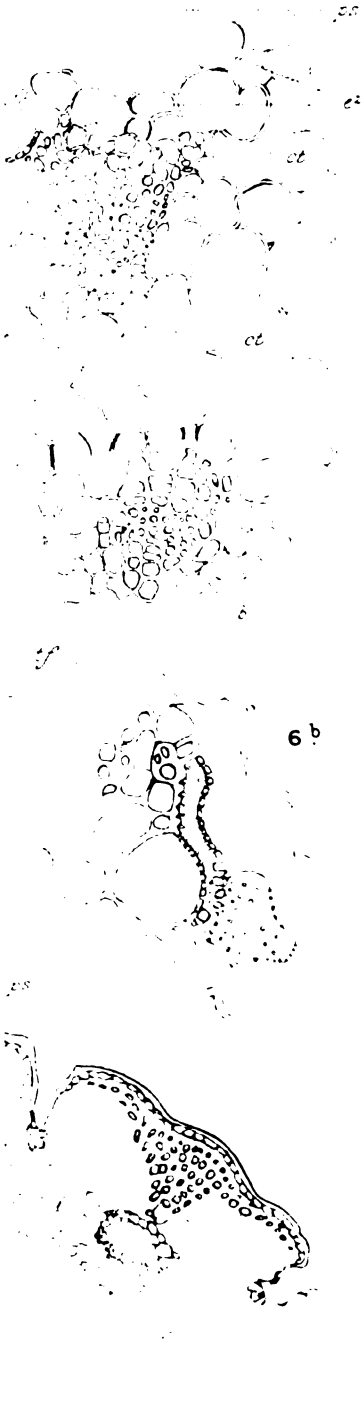
BOODLE & WORSDELL. — CASUARINA.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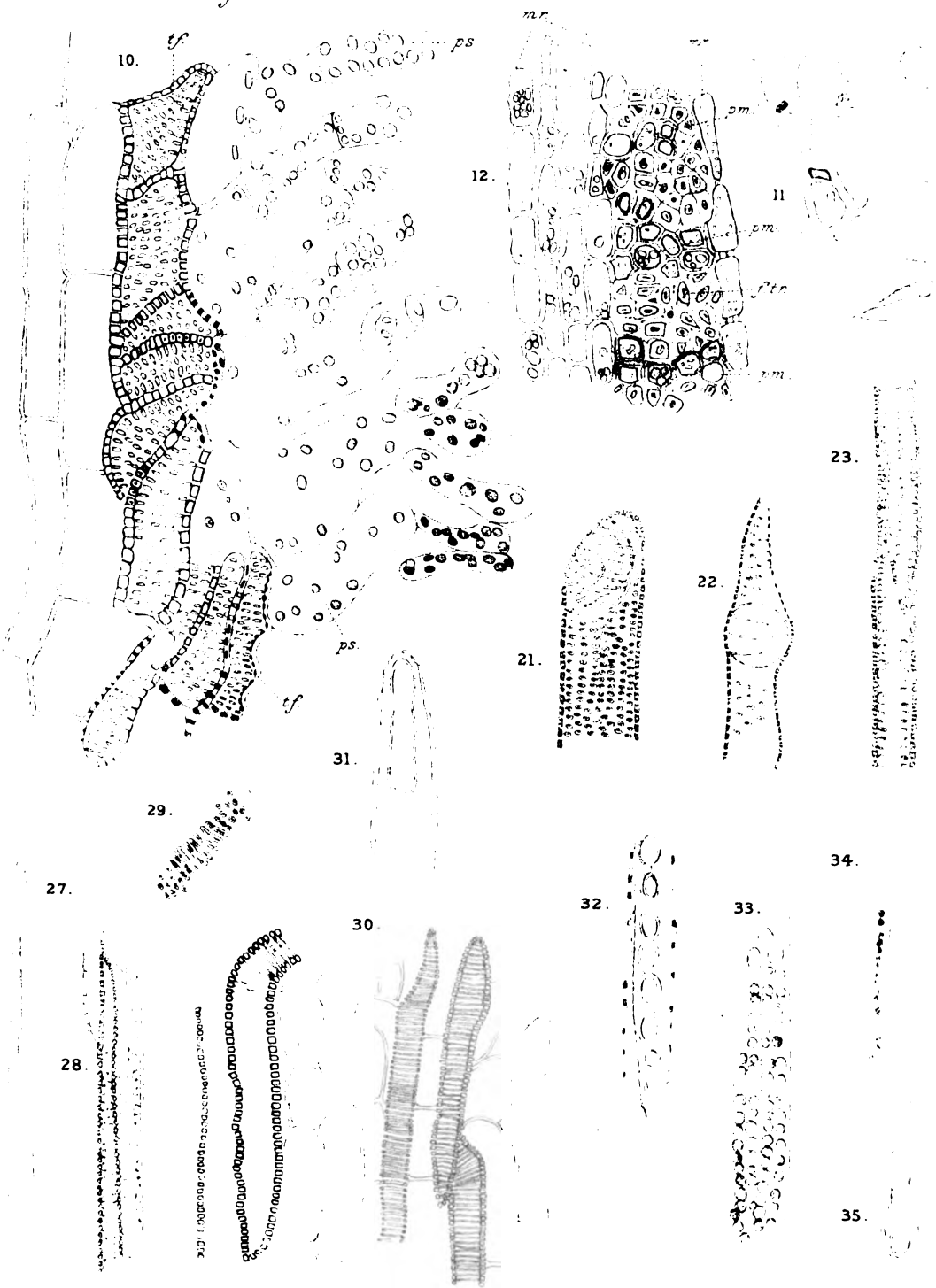

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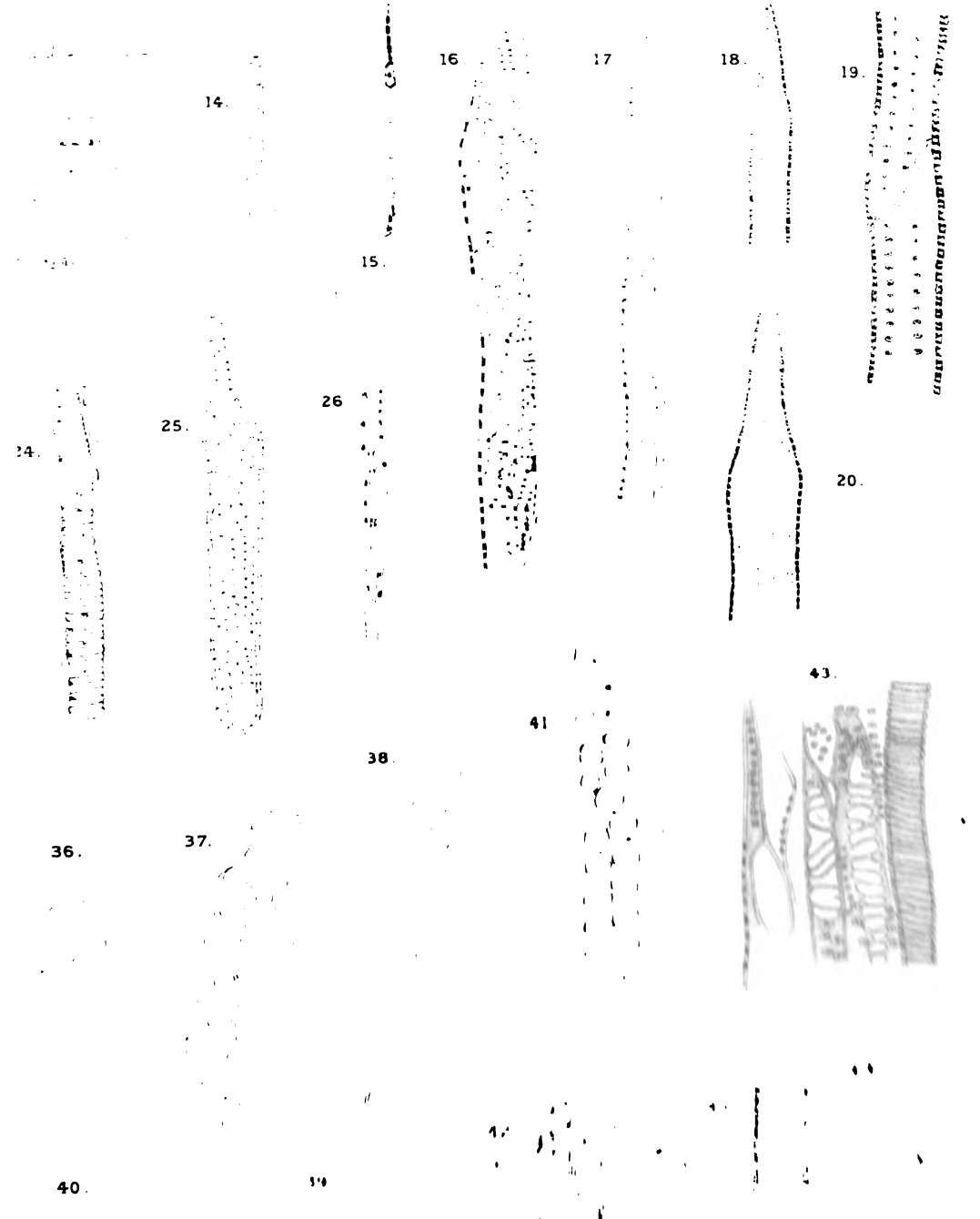
BOODLE & WORSDELL. — CASUARINA.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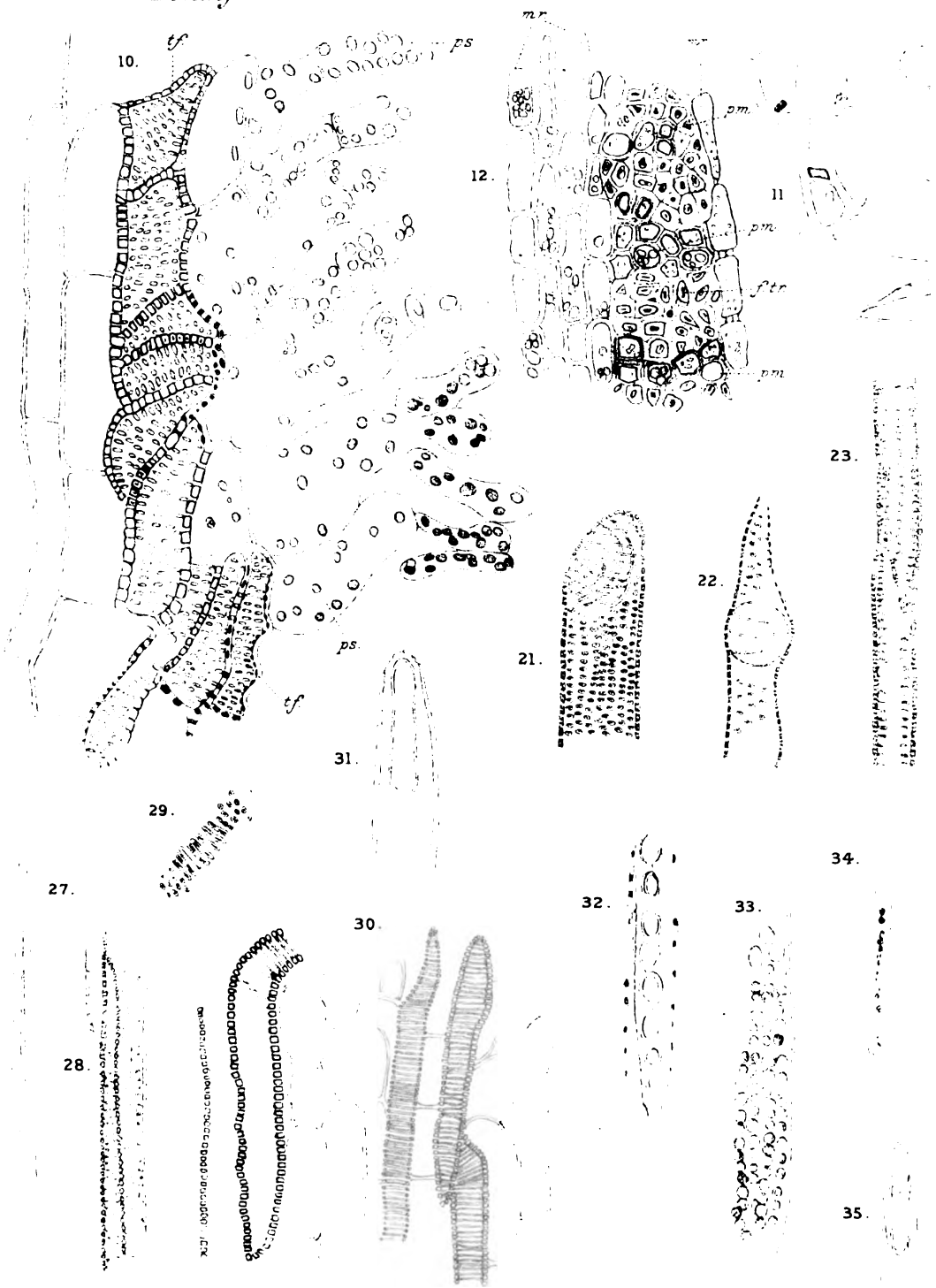

W. C. Worsdell del.

BOODLE & WORSDELL. — CASUARINA.

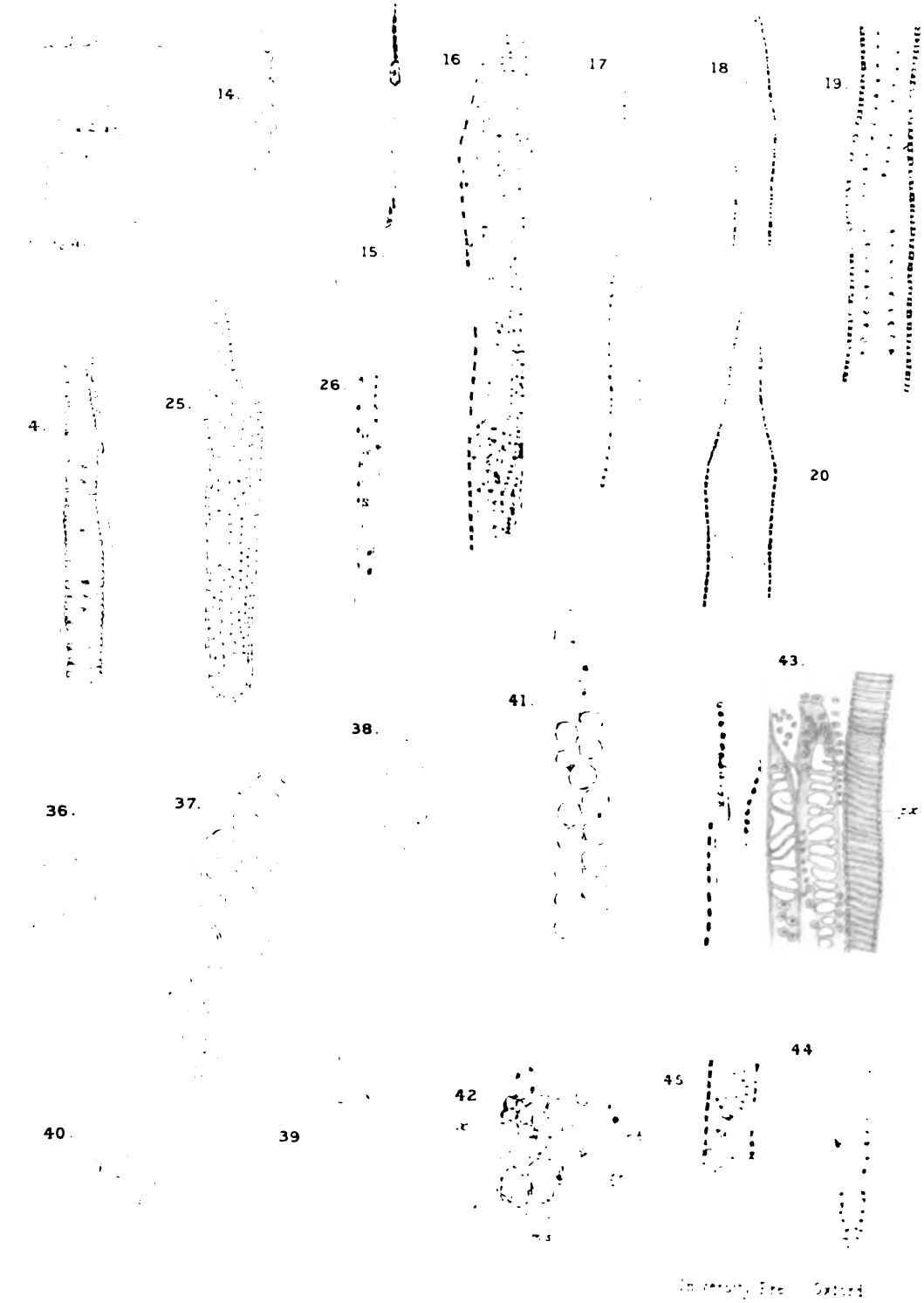

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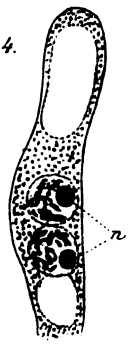
Fig. 1.

Fig. 2.

Fig. 3.



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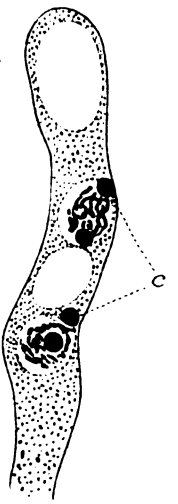

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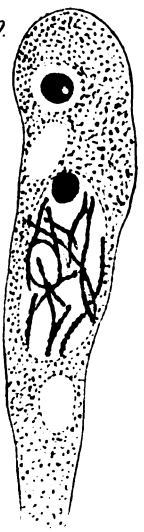

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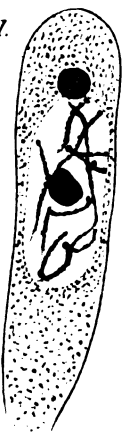

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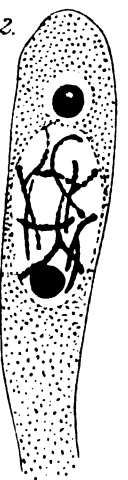

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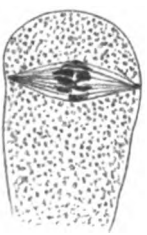

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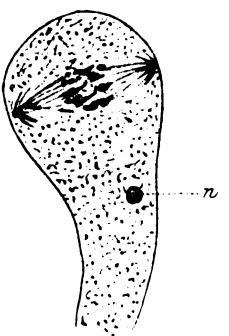

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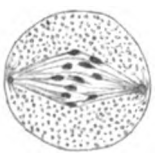

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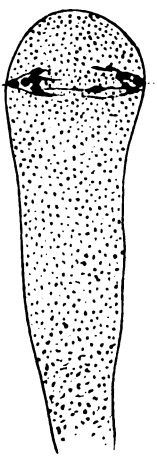

Fig. 20.

Fig. 22.

Fig. 21.

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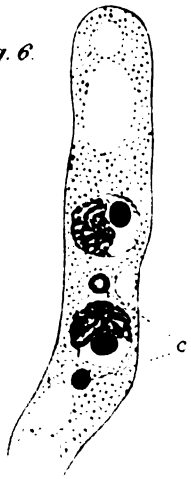
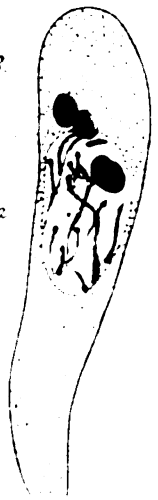


Fig. 7

Fig. 7a

Fig. 8.



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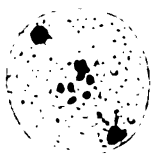
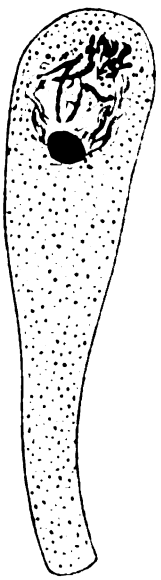


Fig. 14.



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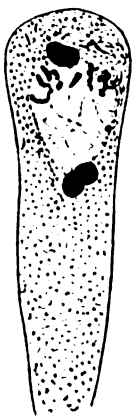

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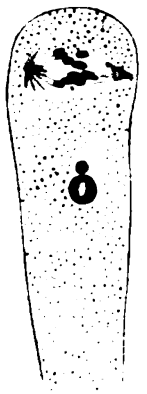

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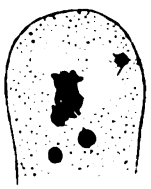

Fig. 24.

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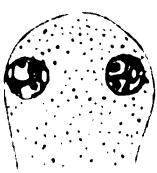
Fig. 28

Fig. 23.



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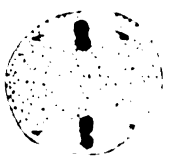
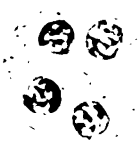
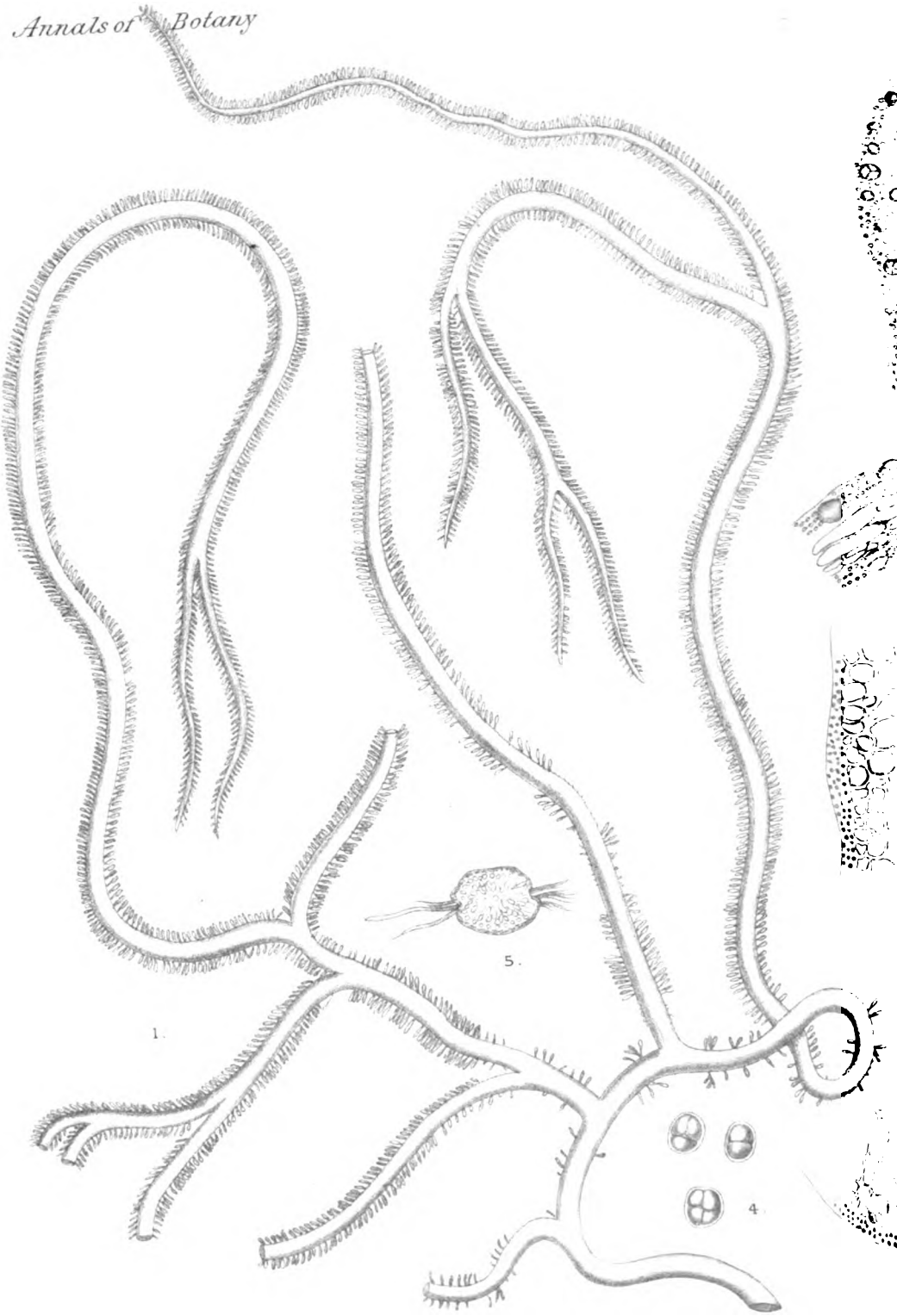


Fig. 27

Fig. 29





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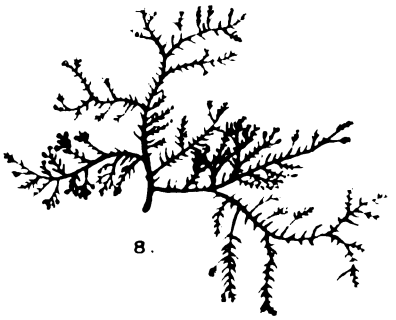

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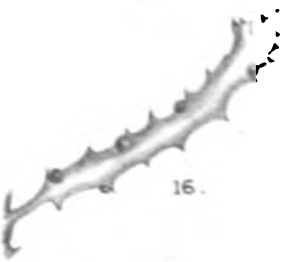

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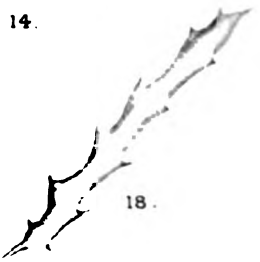

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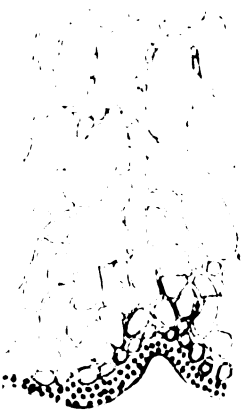

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20. × 200

17. × 50

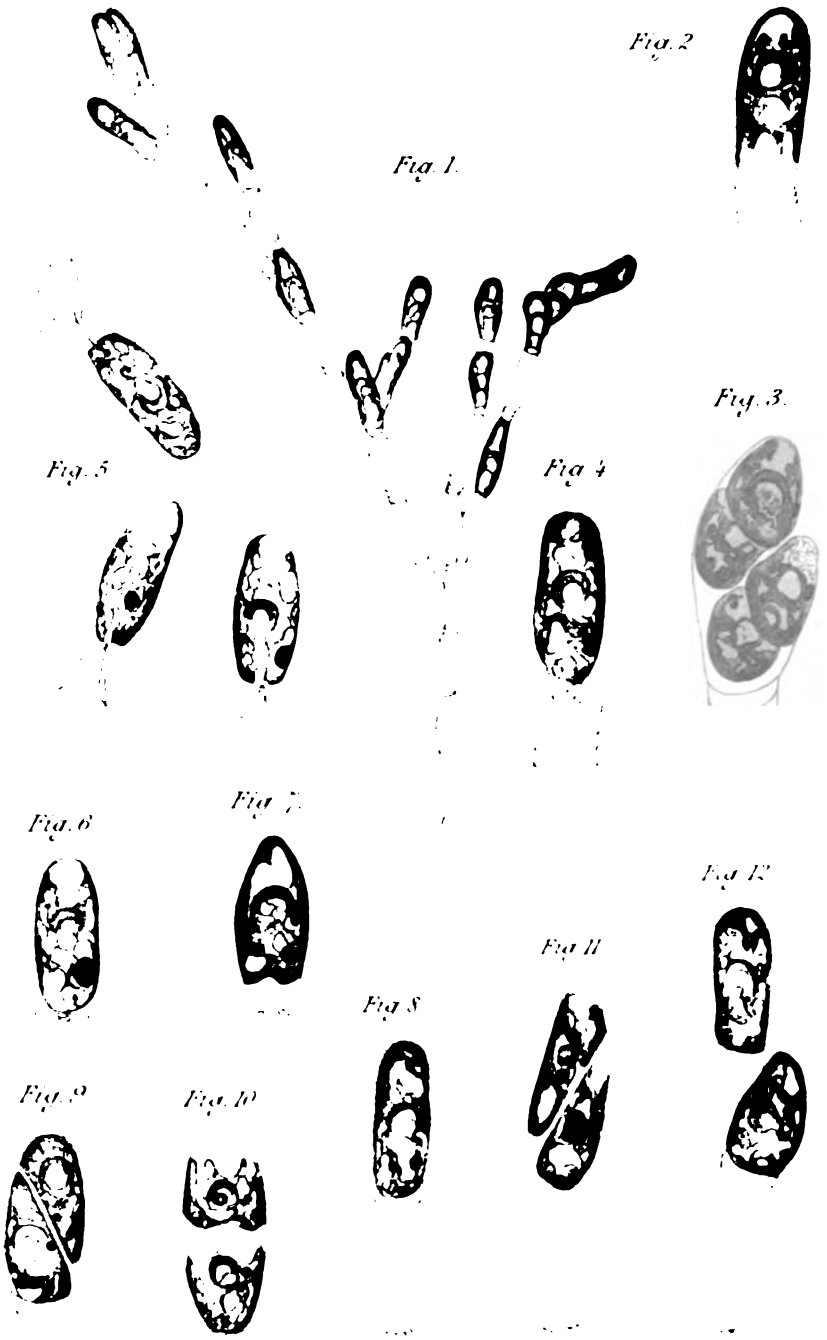
Fig. 1.
Fig. 2
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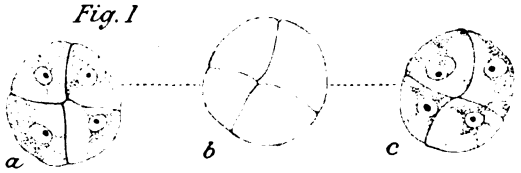
Fig. 1



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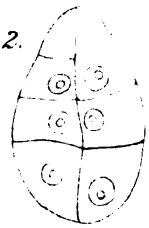

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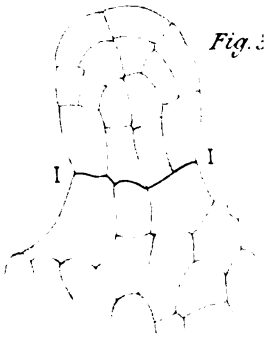

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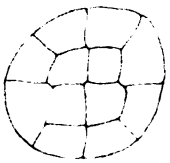

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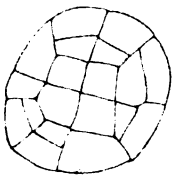

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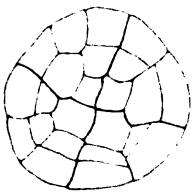

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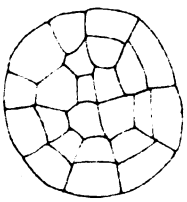

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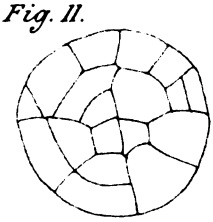

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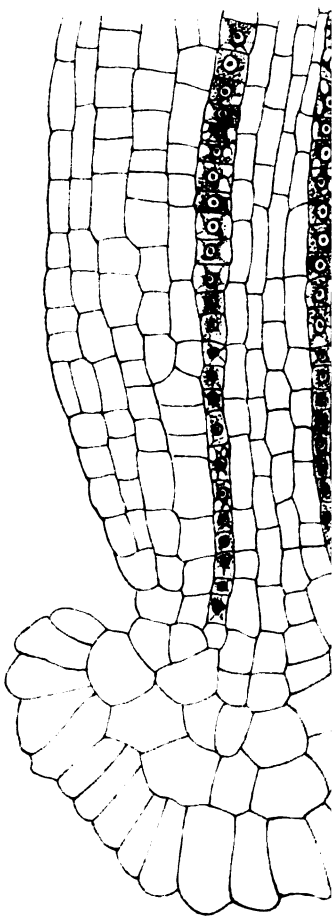

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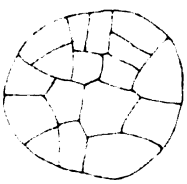

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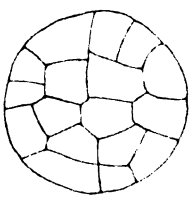

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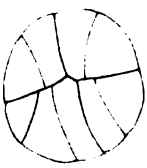

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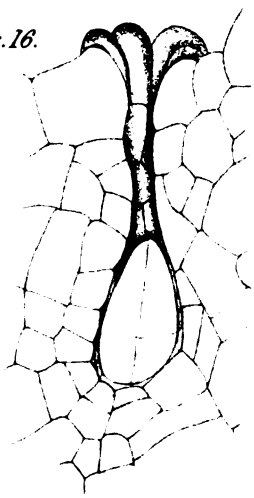

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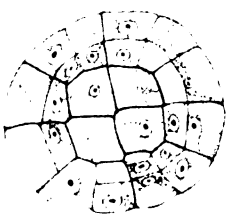

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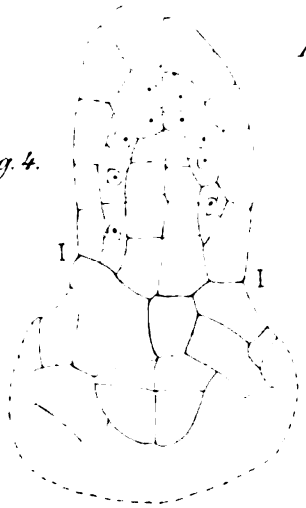

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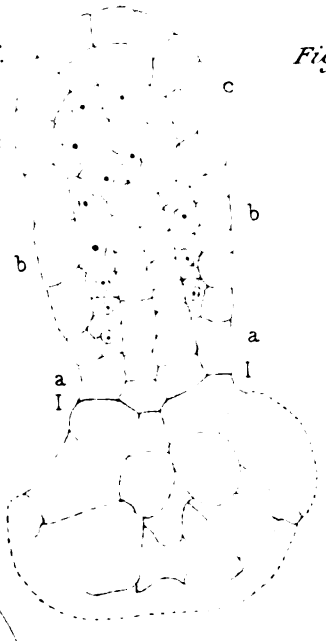

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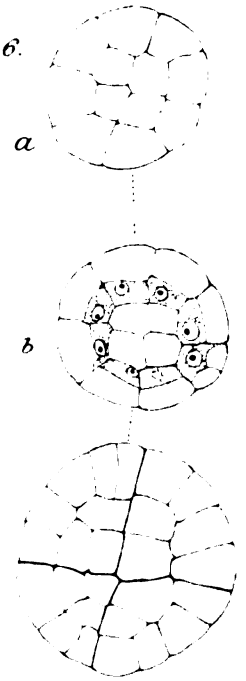

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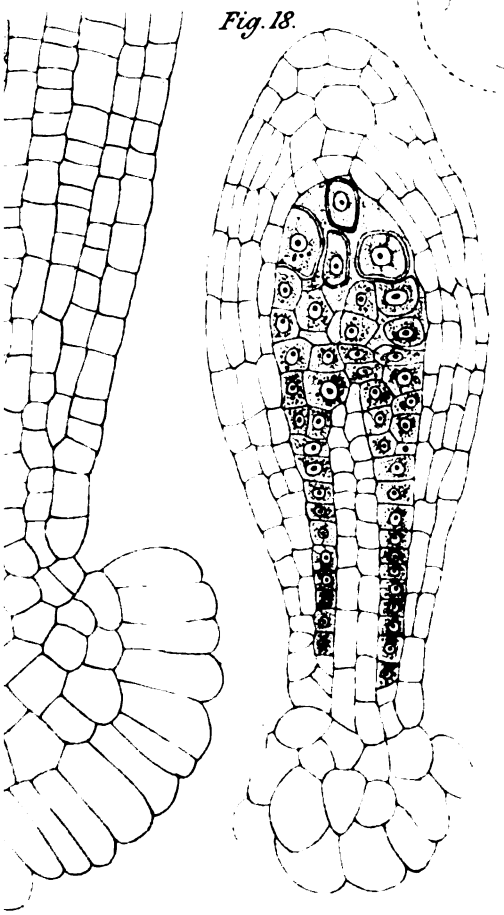

Fig. 17.

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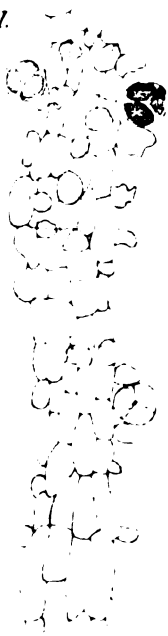
Fig. 20.

Fig. 21.



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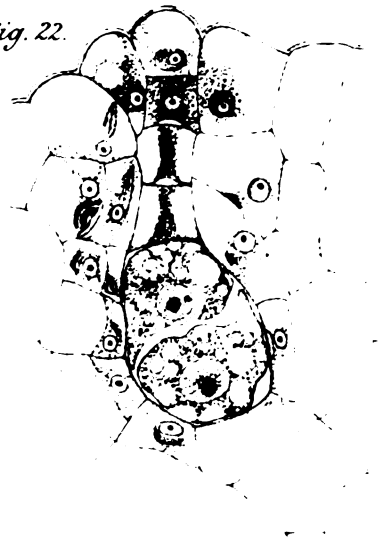

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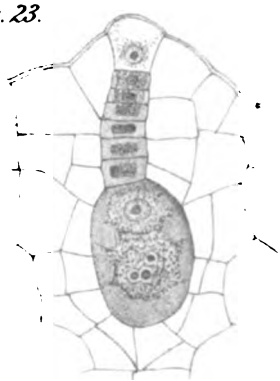

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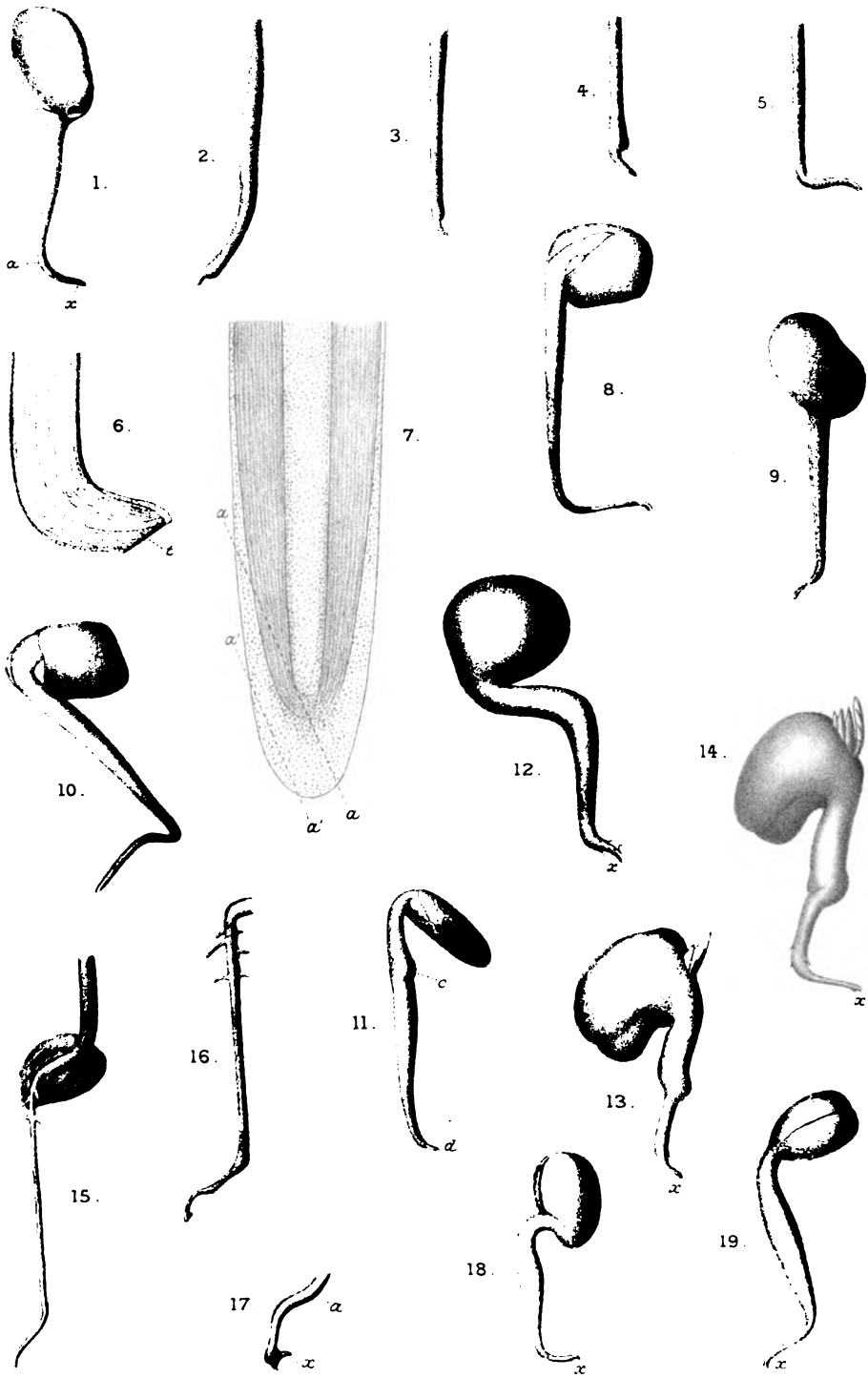

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SPALDING.—TRAUMATROPIC CURVATURE OF ROOTS.

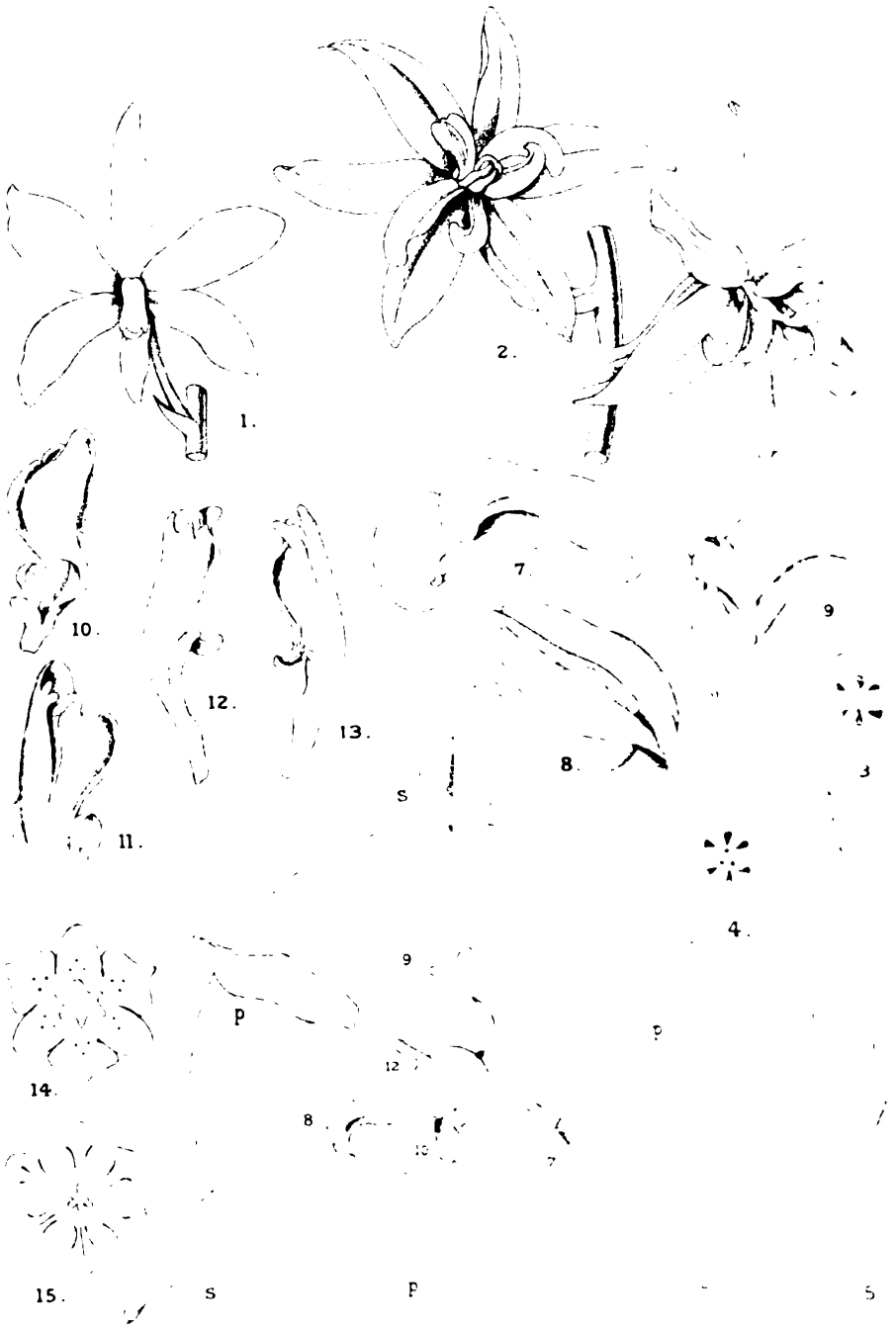

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Fig. 1

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Fig. 3

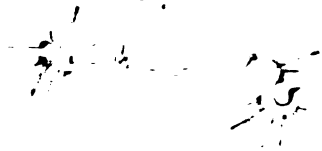
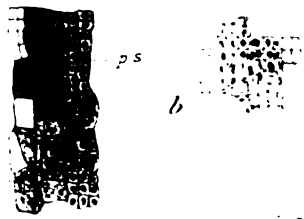


Fig. 4.

Fig. 5



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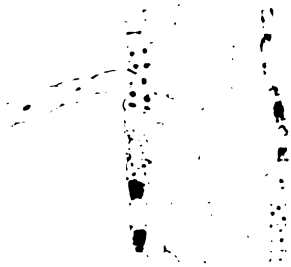

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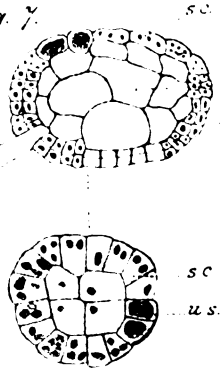
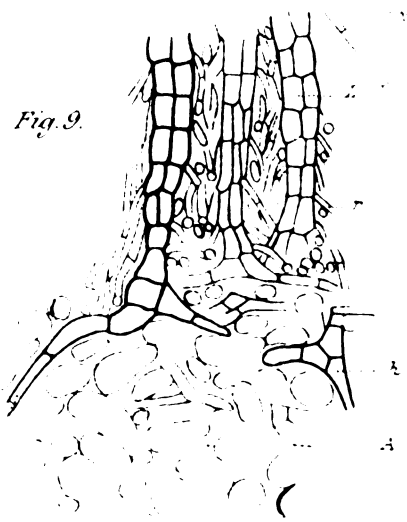


Fig. 8

Fig. 9.



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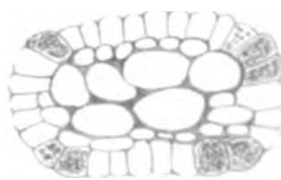

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